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STRAWBERRY XANTHOSIS (YELLOWS), A NEW INSECT-BORNE DISEASE[1]

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INTRODUCTION

Strawberries in California are relatively free from the common diseases of this important small-fruit plant. This is undoubtedly due to the climatic peculiarities of California, and especially to its dry, rainless summers. Because of the low atmospheric humidity during the growing season, powdery mildew and the various fungous leaf spots which are so destructive in humid strawberry-growing sections are of very little importance in California. Also, the damage to the berries from fruit-rotting organisms ordinarily is relatively negligible, except in years of abnormally late spring rains.

By far the most important disease of the strawberry in California is xanthosis, or yellows, which forms the subject of the investigations here reported. In the central California coastal region this disease is so destructive as to be the limiting factor in strawberry growing. The productiveness of the strawberry plantations of this region has been on the decline for a number of years. The length of life of the plantings has also decreased. While formerly it was customary to crop strawberry plantings for 4 years, and 5 and even 6 year old patches were not uncommon, now 3 years is the normal life of strawberry plantations in this region, 4 or 5 year old plantings being very rare. So, during the last 8 to 10 years, the average life of a strawberry plantation in this locality has been reduced by at least 1 year. The quality of the fruit also has become inferior. The cause of this general decline has remained obscure. Red-spider or insect injury, excessive irrigation, or not enough, soil conditions such as alkali, lack of drainage, etc., and variety degeneration, all have been advanced by different growers as possible causes for this decline. Horne (*7*)[3] was the first investigator to recognize the fact that a definite and specific disease was involved and to give a pathological description of it. Just where and when the disease originated is not known. It was first reported from the Watsonville district in 1915

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[2] Grateful acknowledgment is made to R. E. Smith, under whose direction these studies were carried out, for valuable advice and assistance; to W. T. Horne for his sympathetic interest and helpful suggestions; to T. E. Rawlins for advice and assistance in connection with the cytological phase of the work; and to H. H. P. Severin for directing the experiments which established the nonrelation between the causal agents of the strawberry xanthosis and the sugar beet curlytop, and for many helpful suggestions.

[3] Reference is made by number (italic) to "Literature cited," p. 1090.

by H. A. Hyde, who sent specimens of affected plants to William T. Horne. It was in the hope of obtaining some information concerning the nature of the disease that the present investigation was undertaken in the spring of 1924 at the suggestion of Ralph E. Smith.

NAME OF THE DISEASE

In a preliminary report (*12*) the term "yellows" was tentatively applied to this disease. This name is unsatisfactory, however, because it is not specific. A great number of plant diseases are designated by the term "yellows," some of which, like aster yellows and peach yellows, belong to the same class (virus) of plant diseases as the one under consideration, while others (cabbage yellows, for example) are caused by well-known fungous parasites. For these reasons it was decided to name this new disease of the strawberry "xanthosis," the Greek equivalent of yellows.

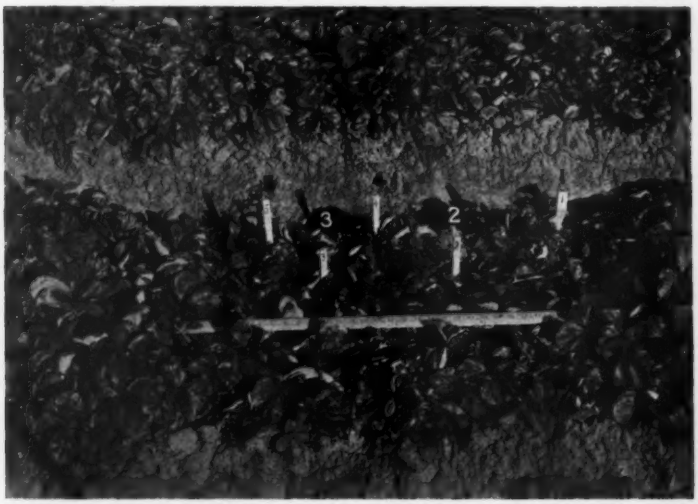

FIG. 1.—Photograph showing the systemic nature of xanthosis. The mother plant (1) is diseased, and so are the four young runner plants (2, 3, 4, and 5) that have come from it

DESCRIPTION OF THE DISEASE

The most conspicuous external symptoms (pl. 1) of xanthosis are: (1) A characteristic crinkling and curling, and usually upward cupping of the leaves. (2) Yellowing of the leaves around the margin and between the larger veins. (3) Dwarfing of the leaves, both petioles and blades. (4) Marked stunting of the growth of the entire plant. (5) Premature reddening, or autumnal color of the older outer leaves. (This is not a very definite symptom, because the leaves of healthy plants also assume a similar color with age or when affected by frost. However, the leaves of diseased plants turn color earlier than those of healthy plants of the same age.) (6) The disease is transmitted through the runners; i. e., every daughter plant arising from a runner from a diseased mother plant becomes diseased. (Figs. 1 and 2.)

Photograph of a young Banner strawberry showing typical xanthosis symptoms Note the small, crinkled, yellow-margined leaves

(7) The first externally visible symptoms manifest themselves in the aerial part of the affected plant; the root system appears per-

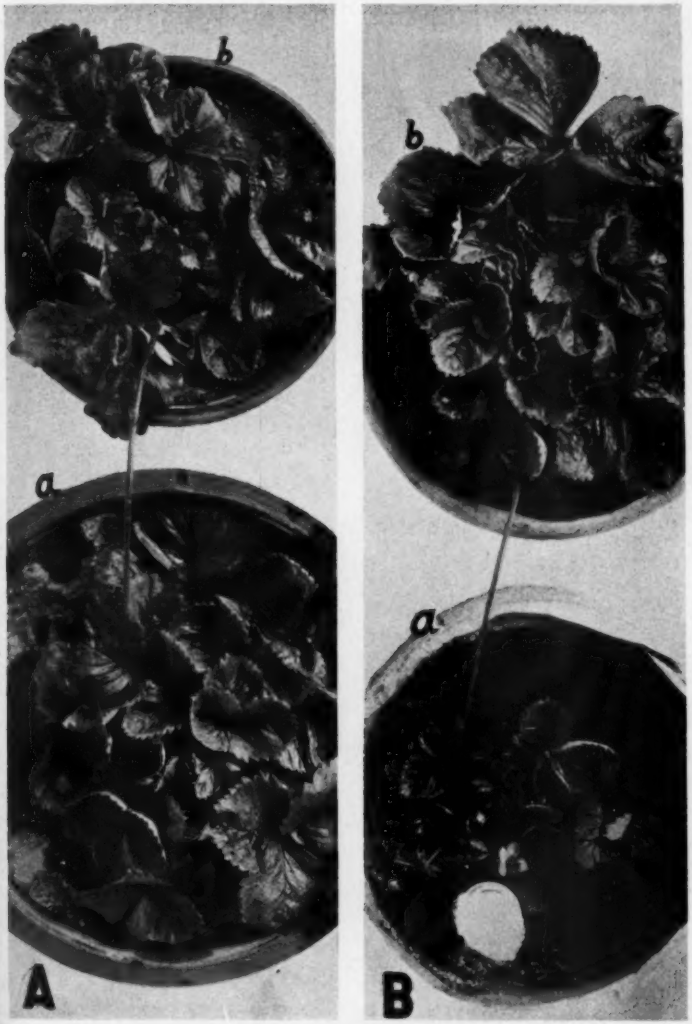

FIG. 2.—Photographs showing both the systemic character of xanthosis and the fact that it is not caused by a soil-inhabiting pathogene. A, Plants in sterilized greenhouse soil; B, sand cultures; *a*, first-generation runners from diseased Banner plant; *b*, second-generation runners. The daughter plants remain diseased even when grown on sterilized soil or sand cultures

fectly healthy during the early stages of the disease. (Fig. 3.) Later on, root decay may set in. (8) The affected plants do not die,

but they never recover, remaining permanently stunted.[4] (9) Leaves that are fully grown at the time of infection remain healthy. Only the very young leaves and those developing after the plant has become infected show the chlorotic and distorted symptoms charateristic of

FIG. 3.—Young typically diseased Banner plant, with normal and well-developed root system, illustrating the fact that the top of the plant first shows the disease symptoms

the disease. (10) The fruit is not directly affected; but because of the reduced vitality of the plant and the smaller leaf area, the fruit is smaller in size and hence is considered inferior in quality.

[4] Dying of individual vines or of several plants in spots in the field is quite a common occurrence, and this dying has been attributed by many growers to the disease under consideration. Although the writer does not know the cause or causes of this dying of strawberry plants, he is satisfied that it is not a phase of the disease under discussion, since it occurs also in localities where the disease has not been found.

The symptoms as described apply to what may be termed "typical" specimens. By this is meant infected Marshall and Marshall-like [5] varieties of strawberries grown under field conditions in the central California coastal region. This definition becomes necessary because under certain conditions the symptoms may become partly or wholly masked. Thus, it was found that if typically diseased plants are grown in the greenhouse under cages at 70° to 90° F., the distortion and curling of the leaves usually disappear, although the other symptoms, stunted growth and a chlorotic condition, continue to persist. Varieties which show a certain degree of resistance to the disease do not exhibit all the typical symptoms even when grown side by side with susceptible varieties.

GEOGRAPHICAL DISTRIBUTION

The disease, while most widespread and most destructive in the central coast district of California, is by no means limited to this region. During the summer of 1925 a careful survey of the strawberry-growing sections of California, Oregon, and Washington was made. In California the disease was not found south of the Salinas Valley on the coast or south of Fresno in the interior. It was found, however, to a limited extent in the San Joaquin and Sacramento Valleys, in Placer County, and in the Sacramento River canyon—in fact, wherever the Banner or Oregon varieties of strawberries are grown. It was also found in Oregon and Washington. Whether it occurs in other parts of the country is not known. It has never been reported from any of the other strawberry-growing States. In England there are apparently several obscure strawberry diseases, variously described by different investigators as "red plant," "cauliflower," "patch," and "little leaf." While most of the English investigators, Johns (*8*), Lees and Staniland (*10*), and Ballard and Peren (*2*), are apparently satisfied that these different terms are synonyms for the same disease, caused by the eelworm *Aphelenchus fragariae* Ritz., others, Turner (*17*) and Beaumont (*3*), entertain doubts as to the cause and suggest the possibility of their being diseases of the virus type.

EXTENT OF DAMAGE

It is not easy to determine the extent of the losses caused by xanthosis, as accurate statistics are lacking. In the hot, interior-valley strawberry-growing sections the damage is relatively small, for under these conditions the infection does not spread readily and the infected plants do not degenerate very rapidly. In the central coastal region, however, the losses are tremendous. An average estimate of a 50 per cent decrease in productiveness of the strawberry plantings in this region is, perhaps, not at all exaggerated,

[5] The Marshall is, of course, a well-recognized horticultural variety (*6*), which has been under cultivation since 1890. The Oregon, too, has been recognized as a distinct variety (*6*). In California one of the chief commercially grown strawberries has also been accepted as a distinct variety under the name "Banner." These three varieties are so similar, both in their vegetative growth and in the appearance of their fruit, that there is a question in the minds of many horticulturists and practical growers whether they are in reality distinct varieties. In addition, as if to make the confusion more complete, several local names—Oregon Improved, New Oregon, New Oregon Improved, Oregon Plum, Rose, and Tuttle—have been given to these varieties, or to what are claimed to be improved strains of these varieties. So the term "Marshall-like" or "Marshall type" is used in this paper to cover all these closely related varieties or forms.

considering the threefold nature of the damage. First, there is a decrease in the total yield of fruit, due to the stunting and decreased vitality of the plants. Second, because of this decreased vitality and smaller leaf area the fruit is generally smaller in size and therefore inferior in selling value. Third, the life of the plantation becomes shorter. Not infrequently young plantings in the vicinity of old, infected fields become so badly diseased the first year, because of infection during the early age of the plants, that they are plowed up the second year. Such plantings are a total loss, since no fruit is produced the first year of planting.

ETIOLOGY

Since the disease was apparently new, and search in the pathological literature of the strawberry failed to give any idea as to its cause, it was necessary to investigate a number of factors as possible causes in the hope that, by the process of testing and eliminating the pathogenic factor might be discovered. The following hypotheses were considered:

1. That the disease is a direct injury caused by the red spider (*Tetranychus telarius* Linn.)
2. That it is caused by adverse soil conditions, especially by the accumulation of salts in such quantities as to produce "alkali" conditions.
3. That it is caused by some soil-inhabiting organism.
4. That it is caused by an infective virus.
5. That it is caused by what has been termed "lack of periodicity." This last hypothesis was early rejected as untenable, however, since it was observed that it was not necessary for plants to overwinter under the mild California climate in order to become diseased. In the spring of 1925 plants that had overwintered in Michigan, where they certainly had become dormant, as well as plants that had overwintered in Oregon, developed the xanthosis symptoms the first season, when planted in Berkeley in the vicinity of infected plants, just as readily as those that had been growing in California continuously.

In the hope of obtaining a lead for possible lines of attack of this problem, the following preliminary experiments were carried out.

EXPERIMENT 1

From a 2 year-old patch in Mayfield, which showed a high percentage of infection, typically diseased as well as apparently healthy plants of the Banner variety were removed very carefully so as to disturb the root system as little as possible, and transplanted in 7-inch pots as follows:

1. Fifteen diseased plants on soil taken from the field in which they had been growing. Untreated.
2. Same as 1, but the tops of the plants were first dipped in a light oil (Volck) emulsion to kill the red spiders with which the leaves were infested.
3. Fifteen plants on good greenhouse soil.
4. Fifteen apparently healthy plants on the same soil as 1.
5. Fifteen apparently healthy plants on same soil as 3.

The experiment was started on May 20, 1924, and the plants were discarded on July 10, 1925.

No difference was observed during the course of the experiment in the appearance of the originally diseased plants, either between those of lots 1 and 3 (growing on different kinds of soil) or between lots 1, and 2 (dipped and not dipped). Furthermore, the disease gradually developed in the originally normal-appearing plants of both lots 4 and 5, until by the end of August, 1924—i. e., about two months after transplanting—every plant in these two lots had developed typical yellows symptoms.

EXPERIMENT 2

Healthy young Banner plants (first-year runners) were planted in 7-inch pots as follows:

1. Twenty-five plants on soil taken from a diseased patch at San Lorenzo. Untreated.
2. Twenty-five plants on same soil as 1 but after it had been disinfected by thoroughly soaking with formaldehyde solution (1 pound in 4 gallons of water) two weeks before planting.
3. Twenty-five plants on rich greenhouse soil. Untreated.
4. Twenty-five plants on the same soil as 3 which had been disinfected in like manner as 2.

The pots were put in saucers, watered carefully so as to avoid leaching the soil and placed outdoors in the plant pathology garden in Berkeley. There were diseased plants in the vicinity (about 25 feet away), and no effort was made to protect the healthy ones from insects. The experiment was started on July 15, 1924, and the plants discraded 13 months later.

It was thought that if the causal agent was a soil-inhabiting organism, the plants of lot 1 would contract the disease, since this soil came from a heavily infected patch and was not treated in any way, while those of lot 2, grown on disinfected soil, would remain healthy. Furthermore, if the causal agent was not a living organism but some physical or chemical pecularity of the soil, the plants of lot 3, which were grown on a different kind of soil (rich greenhouse soil on which no strawberries had ever been grown), would not develop the disease Lot 4 was used as a check to test any possible effect of the disinfectant on the soil.

The plants in all four lots grew vigorously, producing runners profusely. By the end of September of the first season four plants in lot 2 began to show signs of decline, but none developed typical xanthosis symptoms. All those of the other lots appeared healthy. By the end of May of the following year—i. e., about 10 months after planting—the condition of the plants was as shown in Table 1.

TABLE 1.—*Number of plants of the Banner variety used in experiment 2, treatment of each lot, and number of plants in each lot which showed typical xanthosis symptoms 10 months after planting*

Lot No.	Treatment	Number of plants used in experiments	Number of xanthotic plants
1	Planted on undisinfected soil from heavily infected patch	25	4
2	Same soil as in 1, but disinfected with formaldehyde	25	10
3	Planted on rich greenhouse soil; untreated	25	7
4	Same soil as in 3, but disinfected with formaldehyde	25	5

By the time the experiment was discontinued (August 10, 1925) practically every plant in each lot was showing symptoms of disease. The evidence from these two preliminary experiments, while not final or conclusive, appeared to indicate that xanthosis was caused neither by soil conditions (physical or chemical) nor by a soil-inhabiting organism, nor was it a manifestation of red-spider injury. It was observed that lots 2 and 3, which were situated nearest to a patch of diseased Banner plants, were the first to show yellows symptoms. From this, and also from the general symptomatology of xanthosis, as well as from field observations, the writer was led to suspect that perhaps he was dealing with a disease of the virus type which was transmitted by insects. Subsequent experiments confirmed this suspicion.

Experiments were also carried out to test the three other suspected causative factors, namely, red spiders, adverse soil conditions, and a soil-inhabiting pathogene. Since all these tests gave negative results, and since the evidence establishing the virus nature of xanthosis is, in the writer's estimation, definite and conclusive, it is not considered necessary to give here a detailed description of these experiments. The negative evidence is therefore presented here in a brief form.

EVIDENCE THAT XANTHOSIS IS NOT CAUSED BY THE RED SPIDER

Xanthosis symptoms are distinctly different from those of red-spider injury. Red-spider-affected leaves show irregular, characteristically discolored areas, chiefly on the lower surfaces, produced by the chafing mouth parts of the mites, and gradually turn a brownish color and dry up because of loss of moisture through the injured epidermis, but they never develop the marginal chlorosis, the severe stunting, or the crinkling and cupping which are typical characteristics of xanthosis.

Field observations do not show any correlation between red-spider infestation and prevalence of xanthosis. Healthy plants can be found in the field heavily infested with red spiders; and, conversely, typically xanthotic plants can be found practically free from them.

Diseased plants when kept free from red spiders by spraying frequently with light oil (Volck) emulsion did not recover.

Healthy Marshall plants in cages were heavily infested, artificially, with red spiders. During the four and one-half months that these plants were kept under observation they developed none of the xanthosis symptoms.

EVIDENCE THAT XANTHOSIS IS NOT CAUSED BY SOIL CONDITIONS

In California strawberries are grown only under irrigation. For the most part the matted-row system of planting is used. The vines are planted on ridges about 2 feet wide and about 8 inches high. The mother plants are set in two parallel rows at the edges of these ridges, about 18 inches apart, and the runners are allowed to root very close, 6 to 8 inches apart, so that the entire ridge is occupied by plants, forming a rather dense, matted row. (Fig. 4.) The plants are irrigated by completely filling with water the ditches between the rows. This is done once a week, and sometimes oftener during the

summer. For the central California coast district, where the soil is usually heavy, this method of culture is, perhaps, the most unfavorable one that could be devised. As no provision is made for drainage, the water stands in the ditches until it evaporates, very little being lost through percolation. Cultivation after each watering is practiced usually only during the first season but not during subsequent years, as it interferes with the picking of the fruit. As the fruit pickers walk in the ditches, the soil there becomes badly packed, making for very unfavorable drainage and aeration conditions. A system of culture of this sort naturally favors the accumulation of salts, especially if the irrigation water happens to be saline, and this is often the case. In view of this situation it was at first thought that the disease might be due to these adverse cultural practices, resulting in an unfavorable soil reaction; too high a concentration of some particular injurious substance, like Na_2CO_3, or in a too high

FIG. 4.—A well-cared-for first-year planting, showing the matted-row type of strawberry culture in vogue in California. At the time this picture was taken, in early summer, this patch showed about 10 per cent xanthosis infection; by fall practically every plant had become diseased

total concentration of salts. That a similar view was held by others is seen from a brief popular article (*1*) in which it is stated, more or less as a fact, that this trouble is due to excessive concentration of salts, and the belief is expressed that rainfall may solve the trouble by washing out the salts. Accordingly, during the early summer of 1924, soon after this study was undertaken, representative samples of irrigation water and of soil from healthy and diseased patches were collected and analyzed in an attempt to determine whether or not there was any direct correlation between the reaction of the soil solution and the disease, or between the concentration of a particular salt, or the total concentration of salts in the soil and the disease. Only the ions of the salts that are likely to accumulate in quantities large enough to produce alkali conditions—i. e., Ca, Mg, Na, HCO_3, SO_4, and Cl—were determined.

The results of these analyses failed to show any definite positive correlation between the occurrence of xanthosis and either the reac-

tion (P_H) of the soil solution or the accumulation of salts in the soil. It is true that, for any one particular field, soil samples from spots containing badly xanthotic plants usually showed a higher total concentration of salts than the samples from spots having healthy or only slightly affected plants, but this relation does not hold true when samples from different fields are compared. For example, soil samples from a patch having typically xanthotic plants showed a total salt concentration of 346 parts per million, while samples from another field having healthy plants showed a total salt concentration of 1,200 to 1,400 parts per million.

Futhermore, it was observed soon after the analytical studies were undertaken that xanthosis occurs on soils in which alkali conditions can not possibly exist. For example, it was found on Banner plants in a garden in the Santa Cruz mountains where the soil was very well drained and the irrigation water came from a mountain spring. The disease was also found in Oregon and Washington, in localities where strawberries are grown without irrigation. Even plants that were grown on light greenhouse soil in pots contracted the disease, as already reported in this paper.

It seems probable, however, that alkali conditions may play a contributory rôle by accentuating the symptoms of the disease. The xanthosis symptoms, like those of other diseases of the virus type, are often masked, partially or completely, and become more prominently manifested whenever the normal growth of the plant is interfered with. For example, there were seven apparently normal plants (variety Nick Ohmer, which exhibits a certain degree of resistance to yellows) growing in pots, adjacent to typically diseased Banner plants. As these were not protected from insects, they had every chance to contract the disease, yet they continued to appear perfectly normal for over a year. At the end of this period they were removed from the pots and transplanted to the garden. Within two weeks after transplanting they began to show the yellows symptoms, and have become and remained typically diseased since. Apparently, although these plants were harboring the infective principle, the degree of resistance that this variety shows was sufficient to prevent the symptoms from manifesting themselves as long as the growth of the plants was not interfered with. But when the growth was checked by the injury to the root system occasioned by the transplanting the symptoms immediately developed. Many other similar instances were observed. The prominence and destructiveness of yellows in the central coast district may be due in part to the accumulation of salts, which is favored by the heavy texture of the soils, the salinity of some of the irrigation waters, and the cultural practices in vogue in this region. While this mild alkali condition may not be sufficiently toxic to cause noticeable injury to normal plants, it accentuates the decline of plants that harbor the infective principle.

EVIDENCE THAT XANTHOSIS IS NOT CAUSED BY A SOIL ORGANISM

From the behavior of the disease in the field as well as from the results of the preliminary experiments previously recorded, it appeared unlikely that yellows was caused by a soil organism. The disease spreads with extreme rapidity over the entire field, and not gradually in spots as is the case in most of the plant diseases caused by soil-

inhabiting, root-invading parasites. Furthermore, as stated earlier, the disease manifests itself first in the top of the plant, the root system appearing perfectly normal for some time after the top has become diseased. (Fig. 3.) However, this phase of the problem was further studied from different angles, viz:

1. The root flora. Specimens of healthy and of diseased plants were collected from eight different localities in the State, and their underground parts—roots and crowns—were plated, and pure cultures of the organisms developing on the plates were obtained. In the preparation of the media, the plating of the material, and the isolation of the organisms the usual laboratory technic was followed. The specimens included healthy and diseased plants of all ages, from very old plants with most of their root system black and decaying, to very young runners that had just taken root.

The young, sound roots, whether from healthy or from diseased plants, almost invariably gave sterile plates. Over 150 isolations of fungi and bacteria were made from the older roots, or from partly decayed roots and crowns. No attempt was made to classify the bacteria, or to determine the species of the fungus isolations. The following genera are represented by the fungus cultures: Alternaria, Botrytis, Cephalothecium, Cladosporium, Diplodia, Fusarium, Oospora, Penicillium, Phoma, Rhizoctonia and Verticillium. In addition, several other islolations produced no spores, and have not been determined as to genus. As determined by preliminary examinations, 42 of the isolations belong to the genus Fusarium, and at least 18 distinct forms of this genus are here represented. The flora isolated from healthy plants does not differ from that isolated from diseased ones. In these culture studies no one particular organism was found constantly associated with the xanthosis disease.

2. Runners from healthy plants were allowed to root in pots on soil which had been heavily inoculated with soil from around the roots of typically xanthotic plants. Many dead and decaying roots were also included in the inoculum. The plants remained healthy during the 11 months that they were under observation.

3. A set of 10 runners from xanthotic Banner plants were allowed to root on autoclaved greenhouse soil in 7-inch pots (fig. 2, A), and a second set of similar runners were allowed to root on sterilized sand cultures (fig. 2, B) with Hoagland's solution.[6] After the runners had become established they were detached from the mother plants. Then the secondary runners produced by the daughter plants thus established were allowed to root in sterilized soil as before. This process was carried on in some cases for three generations of runners. Figure 2 illustrates this procedure, *a* showing the first generation runners that came from the original diseased mother plants, and *b* the second generation daughter plants. All show the xanthosis symptoms quite clearly.

All the runners, without a single exception, both on soil and on sand cultures, developed into typically yellowed plants, like the mothers from which they originated, and the plants coming from subsequent generations of runners were also diseased. This is felt to be conclusive evidence that a soil organism is not involved. It is

[6] Hoagland's solution has the following composition: Ca, 159 parts per million; Mg, 54 parts per million; K, 193 parts per million; SO_4, 217 parts per million; PO_4, 145 parts per million; NO_3, 717 parts per million.

true that, notwithstanding all precautions, neither the soil nor the sand cultures could have remained absolutely sterile, yet it is highly improbable that every pot of soil and every sand culture became contaminated with the possible pathogenic organism.

4. Extensive cytological and histological studies failed to reveal the presence of any organism in the tissues of diseased plants. What appears to be a mycorhizal fungus was often found in the fibrous roots of both healthy and diseased plants. This fungus apparently has no causal relation to xanthosis, for it is equally common in the the roots of healthy and diseased plants.

EVIDENCE THAT XANTHOSIS IS NOT TRANSMITTED THROUGH THE SEED

It has been found that xanthosis is not transmitted through strawberry seed. Horne (data unpublished) grew a large number of seedlings from seed of infected plants, and all of these remained healthy as long as they were kept away from infective insects. The writer also grew to maturity over 2,000 seedlings from seed of typically xanthotic Banner plants and all proved to be healthy. The nontransmissibility of xanthosis through the seed is a very important fact, since it offers an easy and certain method of obtaining healthy plants of a variety which has become hopelessly infected. It is true, of course, that strawberries do not breed true; but, by growing large numbers of seedlings, some will be found that approach very closely the characteristics of the mother variety.

EVIDENCE THAT XANTHOSIS IS A VIRUS DISEASE

TRANSMISSION EXPERIMENTS

Insect Inoculation

EXPERIMENT 1

Forty healthy young Banner plants in pots of steam-sterilized greenhouse soil were divided into 5 lots of 8 plants each and treated as follows: Lot 1 was placed in an isolated spot, away from any other strawberry plants, to be used as a control. Lot 2 was placed in another isolated spot and lightly infested with red spiders taken from leaves of diseased plants. Similarly, the plants of lot 3 were isolated and infested each with 10 strawberry aphids (*Myzus fragaefolii* Ckll.) taken from leaves of diseased plants. The pots of lot 4 were placed in a patch of diseased Banner plants and left unprotected from insects. The diseased plants in this patch were heavily infested with aphids. Other insects, as well as red spiders, were found on them. The plants of lot 5 were placed in insect-proof cages in the vicinity of the same patch. The experiment was started in the middle of July, 1925.

Lot 1 (the control) remained healthy. These plants became infested with aphids, white flies, red spiders, etc., but apparently these had not come in contact with diseased plants and therefore were not infective.[7] Lots 3 and 4 (i. e., those infested with infective aphids and those left exposed to infective insects, respectively)

[7] The term "infective" is used for the sake of convenience to denote all insects that are known to have fed upon diseased plants, and not merely those that have been proved by tests to carry the infective principle.

began to show xanthosis symptoms about one month after inoculation and developed all the typical symptoms of the disease after another month.

Six of the eight plants that were placed in insect-proof cages (lot 5) failed to grow (a severe treatment with nicotine-sulphate oil emulsion at the time of planting proved too strong for them), but the two that survived developed normally and remained healthy.

One month after inoculation three of the eight plants of lot 2 (those infested with red spider) showed symptoms of the disease; after another five weeks, all were diseased. Aphids appeared on all the plants of this lot soon after the first three plants had begun to develop yellows symptoms, and it seems probable that these aphids inoculated the remaining five plants from the first three. It was at first thought that the red spider transmitted the virus to the three plants which first became diseased. Since, however, in all subsequent experiments the red spider consistently failed to transmit the disease, it is now believed that such was not the case. In October of the same year, a careful inspection was made of the nursery from which the plants were received, and a small amount of xanthosis infection was found there; it is therefore possible that these three plants of lot 2 were actually harboring the infective principle even though they did not show symptoms of the disease at that time.

After this experience, it seemed no longer possible to trust Banner variety plants grown in California or Oregon nurseries for experimental purposes, even when they appeared perfectly healthy. In all subsequent transmission experiments Marshall plants from the Eastern States, where the xanthosis disease has never been reported were employed, or, better still, Banner seedlings that had been kept under insect-proof cages from the time of the germination of the seed.

EXPERIMENT 2

During the summer of 1926, essentially the same experiment as that recorded above was repeated, with the following modifications: (1) Healthy Marshall plants from Michigan instead of Banner were used; (2) an additional control was provided by a lot of eight plants which were infested with 10 noninfective aphids;[8] (3) the aphids, both infective and noninfective, were allowed to feed on the plants for 10 days, after which they were killed by dusting the plants with a dust containing 4.5 per cent of nicotine sulphate. This was done in order to differentiate between the direct injury of the insects and the effect of the virus.

The plan of the experiment and the results obtained are summarized in Table 2. The results verify those of the previous year (experiment 1 in that they show the ability of the aphids to transmit the causal agent of xanthosis, but give negative evidence for the red spider. This is true, also, of all subsequent experiments.

[8] It was found by tests that all strawberry aphids do not carry the infective principle. In fact, it is quite easy, apparently, to obtain noninfective aphids, for until they have fed on diseased plants, they are clean. There are in the writer's stock three cultures of noninfective aphids, one obtained from strawberries from a garden in Yuba City, a second from a garden in Oakland, and a third from wild strawberries growing on the sands of the beach in San Francisco. A fourth clean culture, from the greenhouse of W. T. Horne, in Berkeley, was lost through accident.

TABLE 2.—*Results of transmission experiment 2 with plants of the Marshall variety from Michigan*

[Planted April 26, 1926; infested with insects June, 7, 1926]

Lot No.	Treatment	Number of plants used	Number of plants diseased	Percentage of infection
1	Control 1.—No insects	8	0	0
2	Control 2.—Plants infested with 10 noninfective aphids	8	0	0
3	Plants infested each with 10 infective aphids	8	8	100
4	Plants infested with red spiders (about 20 individuals for each plant) taken from leaves of diseased plants	8	0	0
5	Pots containing the plants placed in a patch of diseased strawberries and left exposed to insects	8	8	100
6	Control 3.—Plants placed in same vicinity as lot 5, but protected by insect-proof cages	8	0	0

EXPERIMENT 3

In this and all subsequent experiments the plants were carefully guarded against chance infection by being kept in insect-proof cages. The types of cages used are illustrated in Figure 5. For

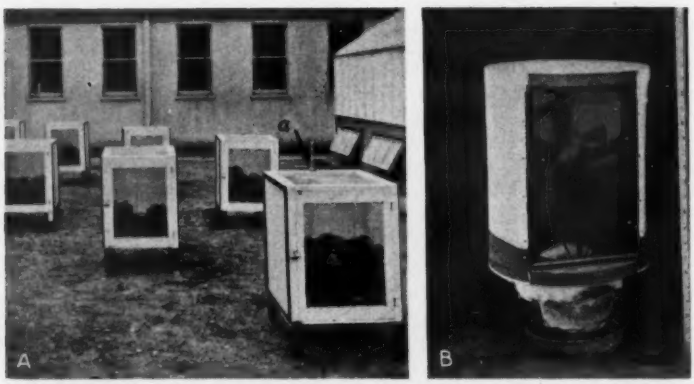

FIG. 5.—Types of insect-proof cages used in transmission experiments: A, large type in which four to six plants may be placed; B, small type for individual plants; *a*, glass tube for watering plants

the small individual cages (fig. 5, B) India lawn cloth was used. The large ones (fig. 5, A) have solid bottoms, glass tops and doors, and three sides covered with unbleached muslin. In both the large and small cages the pots were placed in saucers, and the plants were subirrigated in order to avoid the necessity of lifting the cages to water the plants, thus eliminating the danger of accidental introduction of insects into the cages. The large cages were supported by legs which either rested in pans containing water with a layer of oil, or were coated with tree tanglefoot, so that no ants could crawl in. The doors of these large cages were seldom opened, the plants being watered through a glass tube (fig. 5, A, *a*) which was kept plugged with cotton when not in use. Autoclaved [9] greenhouse soil was employed in all these experiments.

[9] The soil was autoclaved in order to kill weed seed and insects and their eggs.

On July 19, 1926, 21 healthy young Banner seedlings, in individual insect-proof cages since germination, were divided into four sets and treated as follows:

1. Six plants infested each with 15 infective aphids.
2. Five plants infested each with 15 noninfective aphids; control.
3. Five plants infested each with about 20 red spiders from leaves of diseased plants.
4. Five plants infested each with about 20 noninfective red spiders.

The plants thus treated were kept in the greenhouse. The temperature range of the greenhouse was not checked at that time, though it was supposed to run from 70° to 90° F. Later, when there was occasion to make an accurate check of the greenhouse temperature, it was found to reach a much lower minimum. (Fig. 11 and Table 11.) The aphids and the red spiders, both infective and noninfective, were killed after they had been allowed to feed for two weeks, by spraying the infested plants with a dilute nicotine-sulphate oil emulsion.

In about three weeks after inoculation, five of the six plants of lot 1 (infective aphids) began to appear stunted and somewhat chlorotic, but they did not show the typical xanthosis symptoms. Those of the other three lots remained normal. In another four weeks the sickly condition of these five plants of lot 1 became accentuated; the difference between these and the plants of the other three lots which still continued normal, was decidedly noticeable. The stunted growth became more prominent, and the new leaves were chlorotic and progressively smaller in size, but there was very little distortion or cupping of the leaves.

Bearing in mind the well-known influence of high temperatures on the manifestation of symptoms in virus diseases of plants, as well as the results of experiment 4, recorded later, it was thought that this might be a masking of the typical yellows symptoms in the inoculated plants due to the relatively high temperature prevailing in the greenhouse. Accordingly, on August 28, all the plants used in this experiment were removed outdoors, but were still kept under the cages. Within a period of 10 days after removal, the five plants of lot 1 had become typically diseased while the rest of the plants remained healthy. So, apparently, the partial masking of the xanthosis symptoms was due to relatively high greenhouse temperature.

The results of experiment 3 are summarized in Table 3.

TABLE 3.—*Results of transmission experiment 3 with Banner seedlings 5 to 6 months old kept under cages from the time of germination*

Lot No.	Treatment	Number of plants used	Number of plants diseased	Percentage of infection
1	Plants infested each with 15 infective aphids	6	5	83.3
2	Control, plants infested each with 15 noninfective aphids	5	0	0
3	Plants infested each with about 20 "infective" red spiders	5	0	0
4	Plants infested each with about 20 noninfective red spiders	5	0	0

EXPERIMENT 4

Seven healthy young plants (runners from Banner seedlings) in individual insect-proof cages were infested each with 25 infective aphids. Six similar plants were infested each with 25 noninfective aphids to act as controls. The plants of both lots were placed on a low bench (about 8 inches from the ground) on the north side of the greenhouse, where it was relatively cool. The temperature of this spot from August 30 to September 13 is shown in Figure 11 and Table 11. The experiment was started July 15, 1926. The aphids, both infective and noninfective, were killed by dusting with nicotine dust after they had fed on the plants for one week.

Two weeks after inoculation, five of the seven plants infested with infective aphids began to show the yellowing and curling character-

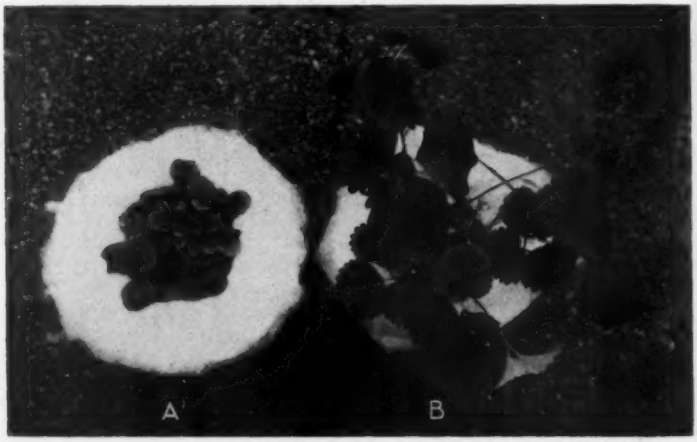

FIG. 6.—A, Banner seedling which became typically xanthotic after being inoculated by means of infective aphids (*Myzus ?raegaefolii* Ckll.): B, control; sister plant of A, infested with noninfective aphids

istics of xanthosis, and within three weeks more they had become typically diseased. (Fig. 6.) The remaining two plants of this lot, as well as those infested with noninfective aphids, remained healthy.

The results of this experiment are summarized in Table 4.

TABLE 4.—*Results of transmission experiment 4*

Lot No.	Treatment	Number of plants used	Number of plants diseased	Percentage of infection
1	Plants infested each with 25 infective aphids	7	5	71.4
2	Plants infested each with 25 noninfective aphids	6	0	0

It should be noted that in this experiment the shortest incubation period (time between date of inoculation and appearance of the first symptoms of the disease) of any of the transmission experiments was observed. It was at first thought that mass infection might be a

factor, since in this experiment a rather large number of aphids (25) were used. This, however, was apparently not the case, for later as many as 50 infective aphids to the plant were used without attaining as short an incubation period as that observed in this experiment. Apparently the conditions of this experiment were favorable for an early manifestation of the symptoms, but as these conditions were not controlled, it was not possible to duplicate them.

EXPERIMENT 5

In this experiment the large cages (fig. 5, A) were used. These were located outdoors and were protected from the north and west winds by a building and the greenhouse, respectively. One cage, containing four young healthy Marshall plants, was used for each lot. At the time of inoculation, these plants were 1 month old from the time of planting, and had from five to seven leaves, all appearing normal. The experiment was started on May 18, 1926. The five different lots were treated thus:

1. Plants were infested each with five infective aphids.
2. Control; each plant was infested with five noninfective aphids.
3. Plants were infested each with about 20 "infective" red spiders.
4. Control; each plant was infested with about 20 noninfective red spiders.
5. Extra control; plants were kept free from insects.

Results similar to those of experiment 3 were obtained. By the end of the first 25 days after inoculation, the plants of lot 1 (infective aphids) had assumed a sickly appearance (stunted growth and partly chlorotic leaves), but they did not show typical xanthosis symptoms. The controls, as well as the red-spider-infested lots, remained normal.

At this time the plants of lots 1 and 2 were reinfested each with 20 infective and noninfective aphids, respectively. These aphids were killed a week later by dusting with nicotine dust. The red-spider-infested plants were sprayed with 1-100 oil (Volck) emulsion to kill the red spiders.

The sickly condition of the plants of lot 1 became more pronounced during the summer; still, the typical xanthosis symptoms did not develop. The leaves were small and chlorotic around the margins, but there was practically no crinkling of the leaves. The control plants and those infested with red spiders remained healthy.

The temperature of the air inside the big cages was recorded for the two weeks from May 31 to June 13 by placing a thermograph therein, and it was found to be high, reaching a maximum of 98° F. It was thought, therefore, that this apparent partial masking of the symptoms might be due to the high temperature prevailing in the cages. Accordingly, on October 2, two of the four plants of lot 1 were removed from the large cage, covered with small individual cages, and placed in a cooler place (on the same bench as the plants of experiment 4). Within two weeks these plants developed typical xanthosis symptoms. So, in this case also, as in that of experiment 3, the high temperature was apparently the factor which partially masked the symptoms of the disease. Table 5 gives a convenient summary of the results of experiment 5.

TABLE 5.—*Results of transmission experiment 5*

Lot No.	Treatment	Number of plants used	Number of plants diseased	Percentage of infection
1	Plants infested with infective aphids	4	4	100
2	Control plants infested with noninfective aphids	4	0	0
3	Plants infested with "infective" red spiders	4	0	0
4	Control plants infested with noninfective red spiders	4	0	0
5	Extra control plants free from insects	4	0	0

EXPERIMENT 6

This experiment was undertaken to test further whether the red spider may act as a vector for the infectious principle of xanthosis. The plan of the experiment was as follows:

1. Six healthy young plants of the Marshall variety were infested each with 20 to 25 "infective" red spiders, that is with red spiders taken from leaves of diseased plants.

2. Control: Six healthy young plants of the Marshall variety were infested each with 20 to 25 noninfective red spiders.

3. Extra control: Three plants infested each with 10 infective aphids. This additional control was used to determine whether the conditions of the experiment were favorable for the development of the symptoms of the disease.

This experiment was started on September 7, 1926, and was continued for two and a half months. The plants were kept under individual small cages outdoors in the same place as those of experiment 4.

Both sets infested with red spiders remained healthy, while the three plants of the additional control (infective aphids) became diseased. So, from the results of this and previous experiments, it is reasonably safe to state that *Tetranychus telarius* Linn. is not an agent of transmission for the infective principle of xanthosis.

MECHANICAL INOCULATION

PRELIMINARY EXPERIMENT

In the summer of 1925 a preliminary experiment was tried to test the transmissibility of xanthosis by inoculating plants with juice extracted from crushed leaves of diseased plants. Eleven apparently healthy Banner plants were inoculated, by means of a hypodermic syringe, with extracted juice from leaves of diseased plants, a second lot of 11 plants were inoculated in a similar manner with juice extracted from leaves of healthy plants, to act as controls, and a third lot of 11 plants were injected with sterile water. The plants used had been in the vicinity of diseased plants for some time and were not protected against insects.

Nine out of 11 of the plants inoculated with diseased juice developed xanthosis symptoms within two months after inoculation, as against 4 out of 11 for each of the two control sets. Although no great importance could be attached to these results, since the plants were left unprotected against infective insects, it was felt that they might

be of some significance. The following two experiments, performed under carefully controlled conditions, gave negative results and failed to verify this supposition.

EXPERIMENT 7

The plants used in this experiment were healthy young plants from runners of Banner seedlings, kept under cages since germination. The juice was extracted in the following manner: The leaves were thoroughly macerated in a mortar, a little water was added, and after continuing the maceration a little longer so as to mix the water with the crushed tissue, the liquid was strained through cheesecloth. The highly turbid liquid was allowed to settle for about five minutes, after which the supernatant liquid, still cloudy but free from large particles, was drawn into the barrel of a hypodermic syringe and immediately injected into the leaves, petioles, and crowns of the plants. The juice was not filtered, for it was decided simply to test its infectiousness first, and if the results of the inoculation were positive, then to test the filterability of the infectious principle. In addition to the thorough injection of the juice by means of the hypodermic needle, some of the leaves of the inoculated plants were mutilated by crushing them between the thumb and the forefinger and rubbing on the injured tissue similarly crushed leaves of diseased plants. To make the inoculation still more thorough, small slits were made in the petioles of the leaves and some of the macerated diseased leaf tissue was inserted. Six plants were inoculated in the manner indicated, while a second lot of four plants were similarly treated, but with juice and crushed tissue from healthy leaves to act as controls. The healthy leaves came from Banner seedlings that had been kept under cages since germination. Both sets were placed outdoors, in cages of the type shown in Figure 5, A. The inoculations were made on May 27, 1926, and the experiment was continued throughout the entire summer.

Both the inoculated and the control plants remained healthy.

EXPERIMENT 8

Because of the relatively small number of plants used in the preceding experiment, it was decided to repeat it. A lot of nine plants (four Marshall and five Banner seedlings) were divided into two lots, as follows:

1. Five plants, two Marshall and three Banner seedlings, were inoculated with diseased juice and macerated tissue.
2. Control. Four plants, two Marshall and two Banner seedlings, were inoculated with healthy juice and macerated tissue.

The extraction of the juice and the inoculation were done in the same manner as in experiment 7. The plants were covered with the small individual type of cages (fig. 5) and were placed outdoors. The experiment was started on September 9, 1926.

This experiment like the previous one gave negative results. Table 6 summarizes the results of the two experiments.

TABLE 6.—*Results of juice inoculation experiments 7 and 8*

Lot No.	Treatment	Number of plants used	Number of plants diseased
1	Plants thoroughly inoculated with unfiltered juice from diseased leaves, with macerated diseased tissue, and by leaf mutilation	11	0
2	Control: Treated like lot 1, but with juice and tissue from healthy leaves	8	0

Although the number of plants inoculated is not very large, it is believed that the results obtained are sufficient to justify the conclusion that the causal agent of xanthosis is not readily transmissible by direct juice inoculation. It may be possible, of course, to obtain a small amount of infection by this method if a large number of plants are used. Instances are known of typically insect-transmitted virus plant diseases in which a small percentage of infection was secured by means of direct juice inoculation. Severin (*14*), for example, succeeded, after other workers had failed, in transmitting the curly-top disease of the sugar beet to 9 out of 100 beets inoculated directly with expressed juice from leaves and roots of diseased beets. The fact that xanthosis is not transmitted by direct juice inoculation is not only of theoretical interest, but has a practical significance to strawberry growers. As the roots and tops of the young plants are trimmed before planting, if the disease were easily transmitted by direct mechanical inoculation, there would be danger of spreading the infection from the few diseased plants that are usually contained in each lot to the healthy ones by means of the trimming knives. The results of the two experiments recorded above indicate that such a danger does not exist.

EXPERIMENT 9

Since the disease was found to be easily transmitted by aphids, but apparently not by direct inoculation with juice extracted from leaves of diseased plants, it was decided to test the infectiousness of juice extracted from infective aphids. Accordingly, about 300 to 400 aphids were taken from leaves of diseased plants, placed in a test tube, and thoroughly crushed by means of a glass rod. About 10 c. c. of water was added, and the mixture was stirred. The crushed bodies of the insects were then allowed to settle to the bottom of the tube, and the supernatant liquid was drawn into the barrel of a syringe fitted to a hypodermic needle, by means of which it was injected into the leaves (midribs and petioles) and into the crowns of six healthy young Marshall plants. The inoculation was done very thoroughly. Small wads of cotton were placed at the opening of the needle wounds and these were soaked with the juice used for inoculation. Four plants were similarly inoculated with juice extracted from noninfective aphids and used as controls. The experiment was started on June 23, 1926, and was continued until November of the same year. The plants were covered with cages of the individual type, kept in the greenhouse for two months after inoculation, and afterwards removed outdoors.

Both the inoculated and the control plants remained healthy. These results can not of course be taken as final, since the number

of plants inoculated was altogether too small. The evidence, however, indicates that the disease can not be readily transmitted by inoculating with juice extracted from infective aphids.

Summary of the Results of the Transmission Experiments

For the sake of convenience, the results of the transmission experiments are summarized in Table 7. The results of experiment 1 are omitted, since, as has been explained, there is some doubt as to whether all the plants used in this experiment were originally free from infection. The results of the preliminary juice-inoculation experiment are also omitted as unreliable, since the plants experimented with were exposed to insect infection.

Table 7.—*Summary of the results of transmission experiments 2 to 9*

Treatment	Number of plants inoculated	Number of plants diseased	Percentage of infection
Inoculated with infective aphids	28	25	89.3
Control: Inoculated with noninfective aphids	23	0	0
Inoculated with "infective" red spiders	23	0	0
Control: Inoculated with noninfective red spiders	23	0	0
Inoculated with juice extracted from diseased plants	11	0	0
Control: Inoculated with juice extracted from healthy plants	8	0	0
Inoculated with extract from infective aphids	6	0	0
Control: Inoculated with extract from noninfective aphids	4	0	0

EVIDENCE THAT XANTHOSIS IS NOT CAUSED BY THE SUGAR-BEET CURLY-TOP VIRUS

The question as to whether xanthosis of strawberries and curly-top virus of sugar beets are identical arose, because of the fact that strawberries are grown in the natural and migratory breeding areas of the beet leaf hopper (*Eutettix tenellus* (Baker)) and often in the vicinity of beet fields. Severin (*15*) has demonstrated that a large number of weeds and cultivated plants of many families are naturally susceptible to curly top and become infected by it. Carsner (*4*) has shown that weeds and a few crops are susceptible or nonsusceptible to curly top, and he came to the conclusion on circumstantial evidence that the beet leaf hopper may have transmitted curly top to beans in Idaho (*5*), although he did not see the diseased plants in the field. Recently, McKay and Dykstra (*11*) arrived at the conclusion, chiefly on circumstantial evidence, that western yellow blight of tomatoes is caused by the virus of sugar-beet curly top. Furthermore, the histological pathology of the strawberry affected with xanthosis is very similar (though not so pronounced), to that of the beet affected with curly top. All the evidence at present available, however, indicates that strawberry xanthosis is a disease distinct from curly top of the sugar beet.

INDIRECT EVIDENCE

The symptoms of curly top of the beet are entirely different from those of strawberry xanthosis. It is true, of course, that symptomatology is not an absolute criterion, since the symptoms of a particular disease may vary considerably with different host plants. In the case of the beet curly top, however, there is one symptom—the trans-

parent venation—which is very definite and is invariably exhibited by plants affected with curly top. This feature is entirely absent in the case of xanthosis. Moreover, the beet curly top does not cause very conspicuous yellowing, whereas in the case of strawberry xanthosis, yellowing is the most pronounced symptom. Another piece of circumstantial evidence is found in the fact that the geographical areas where the two diseases are most severe are not identical—in fact they are exactly opposite. In the hot interior valleys of California, where the beet curly top is most severe, strawberry yellows is very mild, whereas in the cool fog belt of the coast the reverse is true.

DIRECT EVIDENCE

Strawberries Nonsusceptible to Curly Top

Different lots of infective beet leaf hoppers were transferred from curly-top beets to healthy strawberry seedlings and permitted to remain there for a period of about six weeks. Noninfective hoppers were allowed to feed on these strawberry plants and were then transferred to healthy beets, but not a single case of curly top developed. It is evident from this experiment that strawberries are nonsusceptible to curly top.

Beet Leaf Hoppers Failed to Transmit Strawberry Xanthosis

Different lots of noninfective beet leaf hoppers after feeding on strawberry plants affected with xanthosis were transferred to healthy strawberry seedlings and then to healthy beets, but no disease symptoms developed either in the strawberry seedlings or in the beets. In another experiment, noninfective leaf hoppers after feeding on strawberries affected with xanthosis failed to produce disease symptoms in the beets. It is evident that the beet hopper is not capable of transmitting strawberry xanthosis.

INFLUENCE OF TEMPERATURE

That high air temperature exercises a great influence on the symptomatology of virus diseases of plants, tending to obscure, partly or entirely, the symptoms of the disease, is a well-known fact—so well known that it is not necessary here to review the general literature on the subject. Because of lack of appropriate equipment (constant temperature chambers) no absolute figures relative to the influence of temperature on the masking of xanthosis symptoms can be given. The evidence here presented was obtained either from field observations or from a limited number of temperature records.

It was observed soon after these investigations were undertaken and even before evidence was obtained of the infectious nature of the disease, that affected plants, in the field, would make a good growth for three to four weeks in early spring collapse rather suddenly during May, rally considerably during the period from about the middle of June to the end of August, and again collapse during the fall. If a curve were plotted to show this rise and fall in intensity of the disease it would be found practically to coincide with the temperature curves (monthly mean and mean maximum) shown in

Figures 7 and 8, with the exception of the early spring period. The latter discrepancy can be explained by the influence of another factor—the beneficial effect of the dormant period of the winter months.

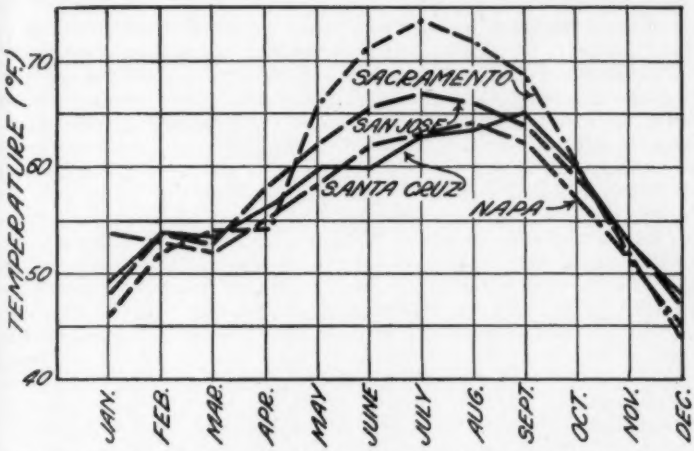

FIG. 7.—Monthly mean temperatures of four different strawberry-growing localities, Santa Cruz, San Jose, Napa, and Sacramento; averages for the two years 1924 and 1925

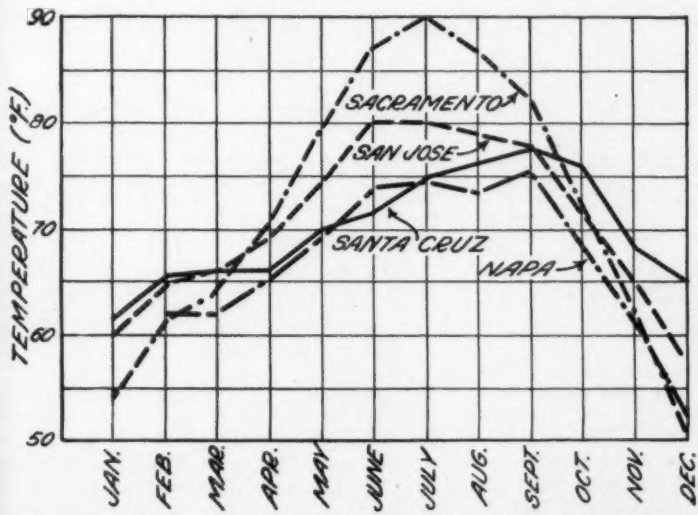

FIG. 8.—Monthly mean maximum temperatures of four different strawberry-growing localities, Santa Cruz, San Jose, Napa, and Sacramento; averages for the two years 1924 and 1925

Horne (7) found that both healthy and sick plants were greatly stimulated by a winter rest period of about two months. It is true, of course, that under the mild climatic conditions existing in Cali-

fornia the plants do not become entirely dormant during the winter, but their growth is markedly checked.

Another significant fact is that while xanthosis occurs in the interior valleys of California, where the temperature is very high during the growing months, it is never serious. The writer had occasion during 1926 to observe a small patch of strawberries at the university farm at Davis. The plants had come from a nursery in which about 10 per cent infection had been found the previous summer. In May this patch was examined and several diseased plants were observed. When examined in August all the plants appeared normal, but on October 24 some showed xanthosis symptoms. Apparently, in this case, the symptoms of the disease were completely masked during the hot summer months. The temperature for Davis is not recorded here, but it is practically the same as that for Sacramento shown in Figures 7 and 8 and in Table 8. The monthly mean and mean maximum temperatures (averages for 1924 and 1925) for four different strawberry-gowing localities, Santa Cruz, San Jose, Napa, and Sacramento are also shown. In the first three localities the disease is very prevalent and very severe; in Sacramento it occurs but is not at all serious. As is shown by the curves in Figure 8, the mean maximum temperature of Sacramento for the summer months is 10° F. higher than that of San Jose, and about 15° higher than that of Santa Cruz and Napa for the corresponding months. The monthly mean temperatures (fig. 7) show a similar relation.

TABLE 8.—*Monthly mean and mean maximum temperatures of four strawberry-growing localities in California for 1924 and 1925*

MEAN TEMPERATURE (° F.)

Locality	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Santa Cruz	49.2	54.3	53.4	55.7	59.6	60.1	62.9	63.4	65.3	52.4	52.9	48.2
San Jose	48.1	54.0	53.0	57.8	61.9	65.5	67.0	65.9	64.0	59.4	53.4	47.2
Napa	53.7	52.8	51.6	55.2	58.4	61.8	63.0	63.6	61.8	57.1	50.7	45.2
Sacramento	46.1	52.1	53.6	53.9	66.2	71.5	73.8	71.6	68.7	60.0	52.4	44.1

MEAN MAXIMUM TEMPERATURE (° F.)

Santa Cruz	61.5	65.4	66.1	66.1	70.1	71.5	74.8	75.9	77.5	76.0	68.5	65.2
San Jose	59.8	64.4	65.7	69.3	74.3	80.1	80.1	79.0	77.7	71.4	65.2	57.1
Napa		61.8	62.2	65.2	69.3	74.1	74.4	73.6	75.6	68.4	61.0	51.8
Sacramento	53.7	61.2	63.7	70.5	79.3	87.0	90.0	86.7	82.2	72.0	61.6	49.7

A further illustration of the masking effect of high air temperature is seen in the following example: In the fall of 1925 four typically diseased Banner plants, in 5-inch pots, were placed in an insect-proof cage, similar to those illustrated in Figure 5, A, but provided with double walls of muslin cloth. The following spring, when growth started, one of these plants showed a very slight yellowing of the margins of the leaves, while the other three appeared entirely normal and remained so during the summer. The temperature of the air in this cage was recorded for a two-week period (May 10 to 24) by placing a thermograph in the cage, and the temperature of the outside air for the same period was recorded by means of another thermograph. These records are shown in Figure 9 and Table 9.

It is seen that the temperature of the air inside the cage reached a point as high as 106° F., while that of the outside air hardly rose above 83°. On May 10 two of the normal-appearing plants were removed from the cage and placed outdoors in a spot away from any diseased plants. To serve as controls, two healthy Banner seedlings which had been kept in a cage similar to that of the diseased ones were likewise removed from their cage and placed outdoors in the same locality as the other two. The outdoor temperature for this period ranged from 48° F. to 78° F. The controls remained healthy throughout the summer. The young leaves of the other two plants began to show a marginal yellowing three days after the plants were

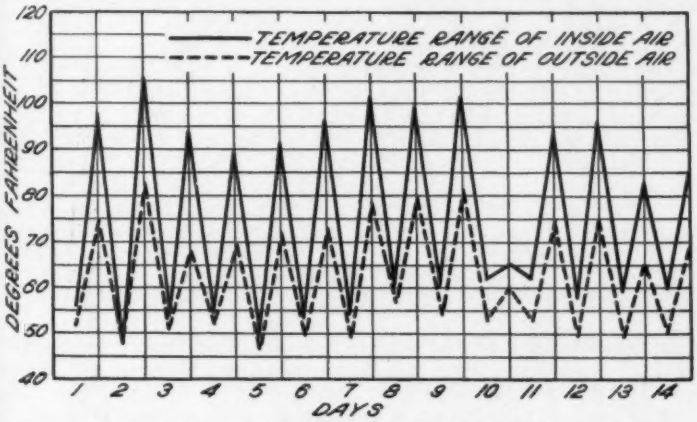

FIG. 9.—Daily range of air temperature inside and outside a double-lined insect-proof cage for the two-week period, May 10-24, 1926

removed outdoors, and all the young leaves subsequently developed exhibited all the typical xanthosis symptoms. So it is apparent that, while the plants continued to harbor the infectious principle, the high temperature within the cage had the effect of masking the symptoms.

TABLE 9.—*Minimum, maximum, mean, mean minimum, and mean maximum temperatures of the air inside and outside a double-lined insect-proof cage, for the two-week period May 10–24, 1926*

Air	Minimum	Maximum	Mean	Mean minimum	Mean maximum
	°F.	°F.	°F.	°F.	°F.
Outside cage	46.0	83.6	61.9	50.1	73.8
Inside cage	48.0	106.0	75.5	55.5	95.5

In discussing the results of transmission experiments 3 and 5, it was stated that the disease symptoms of infected plants were partly masked. A comparison of the daily range of temperature inside and outside the cages used in experiment 5 is shown in Figure 10 and Table 10 and of the inside and outside of the greenhouse (experiment 3) in Figure 11 and Table 11. It is interesting to note that in all these cases where partial or complete masking occurred the

maximum temperature had reached 90° F. or above, and that no masking occurred at a temperature of 84° or below. It is important to consider here that a continuous exposure to high temperature may not be necessary to produce masking effects. Tompkins (*16*), working with the mosaic of potato, found that "relatively short exposures

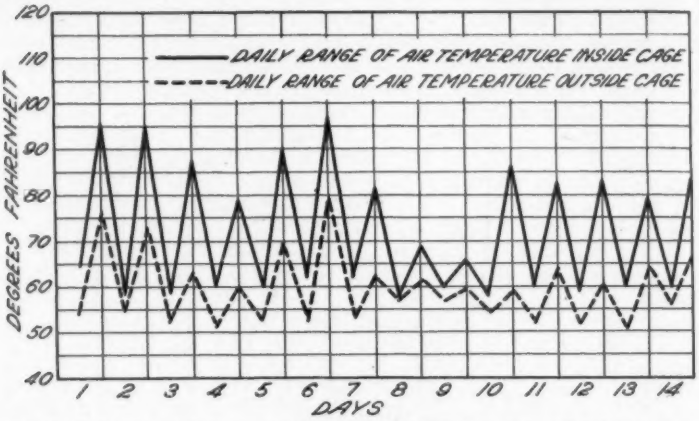

FIG. 10.—Daily range of air temperature inside and outside the large type of insect-proof cages shown in Figure 5, A

to air temperatures above that of the critical air temperature (23° to 24° C.) were sufficient to mask the symptoms of mosaic." Figure 10 and Table 10 illustrate the fact that in studying the masking effect of temperature the maximum rather than the mean should be

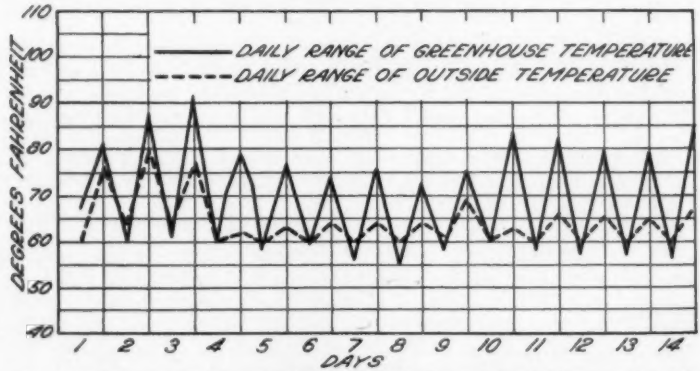

FIG. 11.—Daily range of air temperature inside and outside the greenhouse for the two-week period August 30–September 13, 1926

considered. Thus, for two days, June 8 and 9, the temperature was relatively low (not above 68° F.), so that the mean temperature for the two weeks is relatively low, yet the intermittent exposure to high temperatures was sufficient to partly mask the symptoms. The same fact is illustrated by the curves in Figure 11.

TABLE 10.—*Minimum, maximum, mean, mean minimum, and mean maximum temperatures of the air, inside and outside the large type of insect-proof cages shown in Figure 5, A, for the two-week period May 31–June 14, 1926*

Air	Minimum	Maximum	Mean	Mean minimum	Mean maximum
	°F.	°F.	°F.	°F.	°F.
Outside cage	51	79	59.7	53.8	65.7
Inside cage	58	98	72.7	59.9	85.5

TABLE 11.—*Minimum, maximum, mean, mean minimum, and mean maximum temperatures of the air inside and outside of the greenhouse for the two-week period August 30–September 13, 1926*

Air	Minimum	Maximum	Mean	Mean minimum	Mean maximum
	°F.	°F.	°F.	°F.	°F.
Inside greenhouse	55	91	68.8	58.0	79.6
Outside greenhouse	60	80	64.0	60.4	67.6

Although the evidence here presented does not permit the giving of absolute figures for the critical temperature, it seems to indicate that no noticeable masking of xanthosis symptoms occurs at about 80° F. or below, and that above this temperature partial or complete masking takes place.

CYTOLOGICAL AND HISTOLOGICAL STUDIES

Extensive cytological and histological studies of the different parts of the plant—root, crown, stem, and leaves (petioles and blades)—were made for the purpose of discovering whether or not any organism or any abnormalities could be found associated with the disease. Over 500 slides of fixed and stained material were examined, in addition to a large number of free-hand preparations made from fresh material. For killing and fixing, the alcohol-formalin-acetic acid solution (100 c. c. of 50 per cent alcohol, 6.5 c. c. of formalin and 2.5 c. c. of glacial acetic acid) was used. For the most part the staining was done with Haidenhain's iron-alum haematoxylin, but Fleming's triple stain was also used. The leaves were found rather unsatisfactory for a critical examination of cell content, since the cells contain plastids and other normal structures which stain heavily and obscure the vision.

MYCORRHIZAL FUNGUS IN ROOTS

What appears to be a mycorrhizal fungus has been found almost invariably in the cortex of the fibrous roots, but it has never been observed in the young succulent primary crown roots. This fungus is apparently widely distributed in the soil, for it was found in the roots of plants collected from different fields in widely separated localities in California. Jones (*9*) lists the strawberry as one of the many plants in the roots of which he found a mycorrhizal fungus. Incidentally, the fungus found by the writer seems to be identical with that described by Jones. No attempt was made to isolate and

culture the fungus. Whether it may be considered as a parasite or as a harmless, perhaps even beneficial, symbiotic organism can not be definitely and accurately determined until it has been obtained in pure cultures. The invaded roots appear perfectly normal externally, and no lesions occur in the tissues in which the fungus is found. Certain it is that this fungus has no casual relation to xanthosis. It occurs as commonly in the roots of healthy plants as in those of the diseased.

ABNORMALITIES IN THE LEAVES

The palisade cells of the chlorotic areas of the leaves of diseased plants are noticeably shorter and more compact than those of the leaves of healthy plants, or than those of the green areas of the diseased leaves. This abnormality is apparent both in free-hand sections of fresh material and in fixed and stained preparations.

DARK-FIELD STUDIES

Thin free-hand sections of fresh material—leaves (blades and petioles) and roots—of typically xanthotic plants were examined immediately after cutting with a Leitz dark-field microscope. Also drops of juice obtained by gently rolling the midribs, the petioles, and the succulent crown roots between two glass rods until a drop of the liquid oozed out from the cut end, were placed on slides and examined in the same way. No organism of any kind could be seen either in the cells of the sections or in the extracted juice.

TISSUE DEGENERATION AND CELL INCLUSIONS

A degeneration of the tissue of the pericycle region of the roots, accompanied by certain conspicuous cell inclusions, was found constantly associated with the disease. These abnormalities were so strikingly similar to those described by Rawlins (*13*) for sugar beets affected by curly top that the same terminology is, for the sake of convenience, adopted here.[10] The two types of cell inclusions are therefore designated as x and y bodies, respectively.

The tissue degeneration is chiefly found in the pericycle, but it often extends to the adjacent phloem, and more rarely to the endodermal cells. Figure 12, A and B, illustrates this tissue degeneration, shown by the black-staining groups of cells. What is considered to be the first state in the process of degeneration is that certain pericycle cells become filled with what appears to be reticulate cytoplasm (fig. 13, A, *a*), which later becomes granular. About this time the nucleolus has disappeared, and the nucleus shows decided signs of disintegration. The nuclear membrane appears shrunken, inclosing some amorphous granular material, staining black with Haidenhain's haematoxylin and red with the triple stain. In what is considered to be a later stage in the process of degeneration, the nuclear membrane incloses only a small amount of granular material, and it is either shrunken or very well rounded as if filled with liquid (fig. 13, B, *n*); and the part of the cell normally filled with cytoplasm contains either x or y bodies, or both. The x-bodies (fig. 13, A,) are composed of dense, amorphous material which stains black with

[10] In addition to the abstract cited, through the courtesy of its author, the writer was privileged to read the original manuscript of the yet unpublished complete work of T. E. Rawlins.

haematoxylin and red with the triple stain. The y-bodies (fig. 13, B.) are identical in their staining reactions with the x-bodies, but have a more definite shape than the latter, being spherical or nearly spherical, and usually appearing to possess a distinct membrane. Neither the x nor the y bodies show any organization of structure. In what appears to be the final stage in the process of degeneration, the remnant of the nucleus and the x or y bodies disappear, and the entire cell is filled with amorphous, somewhat granular material, staining black with haematoxylin.

Observation of hundreds of sections has led the writer to believe that the process of degeneration is as follows: In what has been considered the first stage the nucleus is practically intact, though showing signs of degeneration; in later stages the nuclear degeneration has proceeded further, until most or all of the nuclear material

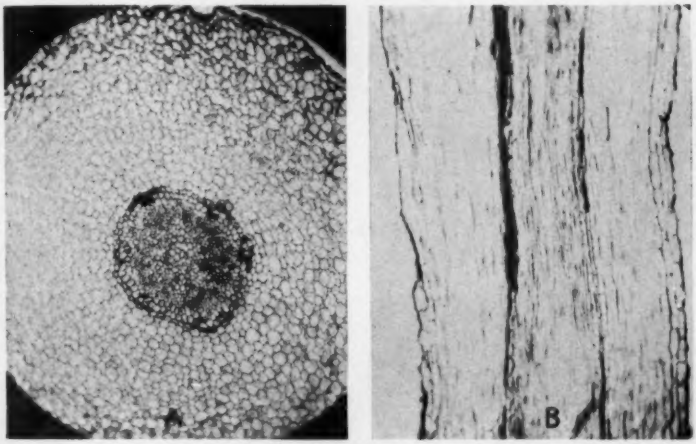

FIG. 12.—A, Cross section of young root of xanthotic Banner strawberry showing degeneration of some pericycle and adjoining cells, × 335; B, longitudinal section of young root of xanthotic Banner strawberry showing tissue degeneration in the pericycle region, × 400

has disappeared from the nuclear membrane, and at the same time the x or y bodies, or both, have made their appearance. No cells have been found containing x or y bodies in which the nucleus was not degenerated. Finally, both the remnant of the nucleus and the x and y bodies become indistinct.

SIGNIFICANCE OF THE ABNORMALITIES

Just what significance may be attached to the abnormalities described, and especially to the peculiar cell inclusions, can not be definitely stated. The fact that these abnormalities have been found constantly associated with the disease, but have never been found in roots of healthy plants, would suggest that they must have some relation to the disease. It is not believed that they bear any causal relation, however. Neither the x nor the y bodies show any structure that would suggest the possibility of their being organisms. They are probably degeneration products of the cell. The fact that

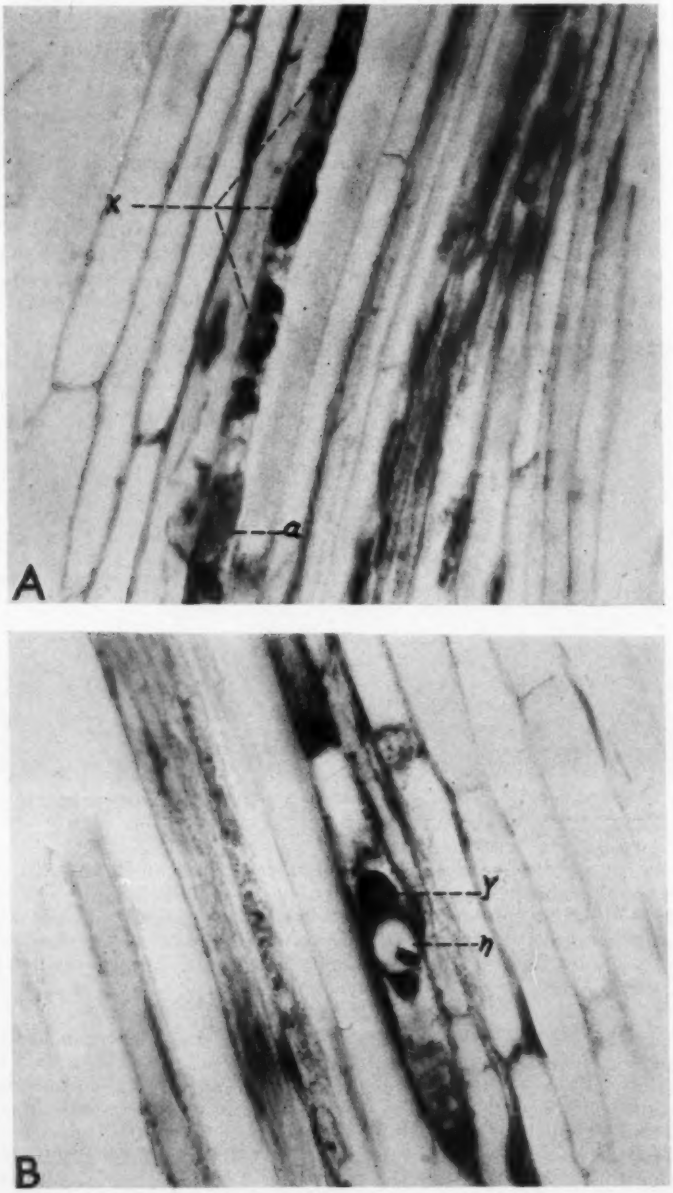

FIG. 13.—A, Photomicrograph of longitudinal section of young root of xanthotic Banner strawberry showing pericycle cells containing x-bodies (*x*) and a cell filled with reticulate, black-staining cytoplasm (*a*), × 570; B, pericycle cell with degenerated nucleus (*n*) and a typical y-body (*y*), × 600

they exhibit the same affinity for stains as the nucleus, and that they make their appearance in the cell simultaneously with the partial or complete disappearance of the contents of the nucleus, would suggest that they may be extruded nuclear material.

The same type of pathological histology and the same type of cell inclusions described here were found by Rawlins (*13*) in sugar beets affected with curlytop. Yet, as is shown earlier in this paper, the evidence indicates that the two diseases, xanthosis of the strawberry and curlytop of the sugar beet, are not identical. If it is correct to consider these cell inclusions as degeneration products, it must be concluded that two distinct, but perhaps similar, causal agents may bring about identical pathological manifestations. Until more is known of the nature of viruses and the mechanism by which they bring about the observed pathological phenomena in plants, any interpretation of the nature of the various abnormalities found must, of necessity, be of a speculative character.

SPECIFIC AND VARIETAL RESISTANCE

An extensive project to test varietal resistance in strawberries is now in progress. The results of these tests will not be known for some time; here are given only the results of the observations of the last two years regarding specific and varietal resistance. Of the cultivated varieties, the Marshall type, in which are included the Marshall, Oregon, Oregon Improved, New Oregon, Oregon Plum, and Banner varieties, is the most susceptible to xanthosis. The Nick Ohmer, the Melinda, and the Superb are slightly more resistant than the Marshall type. The Corsican (Uncle Jim) is interesting in that it contracts the disease very readily and shows the yellow leaf very prominently, but does not become greatly stunted. The following varieties have thus far shown a rather high degree of resistance: Early Ozark, Howard 17, Dr. Burrill, Dunlap, Premier, Parsons, Campells Early, August Luther, and Americus. They are not immune, however, for when exposed to infection they contract the disease, but they do not develop all the typical symptoms. The stunting of the plants, for example, is relatively slight compared with that of the plants of the Marshall type. Of the wild species, the beach strawberry, *Fragaria chiloensis* Duch., is immune to the disease as far as one can judge from external symptoms. Whether or not it can harbor the virus has not been determined. The same is true of practically all of Etter's varieties and new selections. Over 70 selections of these Etter strawberries have been grown for a year in the immediate vicinity of diseased Banner plants, under conditions most favorable for infection, and with the exception of two, all have remained healthy. No transmission experiments were tried to determine whether or not the infective principle is harbored by these normal-appearing selections. As all of Etter's varieties contain, to a smaller or greater degree, the *chiloensis* blood, they owe perhaps their high resistance to their wild parent.

A second wild species, the wood strawberry, *Fragaria californica* C. & S., is apparently susceptible. Plants of this species, growing next to a xanthotic Banner plant in a garden in the Santa Cruz Mountains, contracted the disease. This is the only instance in which xanthosis was found on wild strawberries.

POSSIBLE CONTROL MEASURES

No definite means of control can be given at this time. The work thus far has been chiefly concerned with the etiology of the disease. There are, however, certain methods of attack that readily suggest themselves as control possibilities. From the present limited knowledge of the nature of the causal agent or agents of diseases of the virus type, it is clear that no curative measures can be employed. Preventive methods, however, have been used in controlling diseases of this kind, and in many cases these have been very successful. These methods consist chiefly in the use of planting stock which has been obtained as free from a particular virus disease, or a combination of such diseases, as possible by means of roguing, seed indexing, etc., and in further eliminating the infection by inspecting the plantings several times during the growing season and removing any plant that is found to be diseased.

From observations in the field, as well as from the results of certain experiments which were started last year and are still in progress, it is strongly believed that roguing will prove effective in controlling the xanthosis disease, or at least in greatly checking its spread. There are, however, certain difficulties which militate against an easy application of this method. The most important of these is the fact that, as previously stated, the symptoms of the disease are often completely masked under certain conditions; and, so, in roguing, plants which harbor the virus, but appear healthy, will not be destroyed, and these will serve as a source of infection. The second great difficulty is that even when there is no complete masking the symptoms of yellows are not very sharp and readily defined during the early stages of the disease, and so a certain degree of skill is required to recognize the doubtful cases. But notwithstanding these difficulties it is felt that this method of control is capable of practical application if carried out thoroughly under the supervision of trained inspectors.

Another possible method of control may be suggested here, but what measure of success will attend its use can not even be predicted until more experimental work has been carried out. If it is found that xanthosis is specific as to its vector; that is, if it is transmitted only by the strawberry aphid, it is possible that it might be controlled effectively, indirectly, by spraying or dusting with nicotine, since the aphid is very readily killed by this means.

A more radical means of control lies in the development of resistant varieties. This is a long process, requiring years of breeding and careful selection, but in this case it appears very promising for two reasons: (1) A large number of commercial and semicommercial varieties of strawberries show a marked degree of resistance to xanthosis, and so there is available an enormous amount of valuable material for breeding; (2) the strawberry is an exceedingly convenient plant for breeding, since it is propagated vegetatively. Once the desired cross has been secured, the work is ended; we are not concerned about its genetic constitution, and it is not necessary to continue the breeding until a pure line is obtained, as in the case of seed-propagated plants.

In view of these considerations, an extensive breeding project has been undertaken for the purpose of developing a variety that will

be resistant to xanthosis and that will possess the desirable commercial qualities to meet the agricultural conditions in California.

SUMMARY

This paper presents the results of investigations on the etiology and transmission of a new insect-borne disease of the strawberry (Fragaria). The term "xanthosis" (the Greek equivalent of "yellows") is proposed as a name for this disease, the symptomatology of which is described.

The economic importance and the geographical distribution of the disease are discussed.

Experimental evidence is given which shows that xanthosis is not due to injury from red spider (*Tetranychus telarius* Linn.), to lack of periodicity, to soil conditions, or to a soil-inhabitating pathogene, but that it is caused by some infectious principle of the virus type. This is the first disease of this nature to be reported on the strawberry.

The disease has been successfully transmitted from sick to healthy plants by means of infective strawberry aphids (*Myzus fragaefolii* Ckll.). Aphids that have not fed on diseased plants are noninfective.

The red spider is not capable of transmitting xanthosis.

Mechanical inoculation with unfiltered juice from diseased plants, or with extract from the crushed bodies of infective aphids, as well as by means of leaf mutilation, failed to transmit the disease.

Xanthosis is not transmitted through the seed.

Circumstantial as well as direct evidence is given to show that xanthosis is not caused by the virus of the sugar-beet curly top. The beet leaf hopper (*Eutettix tenellus* (Baker)) was found incapable of transmitting the causal agent of xanthosis either to strawberries or to beets.

The effect of temperature on the masking of the symptoms of the disease is discussed. Air temperatures of about 80° F. and higher induce partial to complete masking of the symptoms.

Extensive cytological and histological studies of the different parts of the plant were made, using fresh as well as fixed and stained material. The findings of these studies are as follows:

1. A mycorrhizal fungus was repeatedly found in the small fibrous roots. This fungus has no causal relation to xanthosis, for it is equally common in roots of healthy and of diseased plants. No other organism was found in any of the above-ground parts of the plant.

2. The palisade cells of the chlorotic areas of the leaves are considerably shorter and more compact than those of the healthy plants or of the green areas of the leaves of the diseased plants.

3. A degeneration of the tissue of the pericycle region of the roots was found to be constantly associated with the disease. In this same region cells were often found with partly or completely degenerated nuclei and containing two types of black-staining bodies, designated in this paper as x and y bodies. These cell inclusions display no internal structure, and are considered to be degeneration products. Neither the degenerating tissue nor the cell inclusions were ever found in roots of healthy plants.

Specific and varietal resistance to xanthosis are briefly discussed. The beach strawberry, *Fragaria chiloensis* Duch., is immune, as far

as can be judged by external symptoms, while the wood strawberry, *F. californica* C. & S., is susceptible. The cultivated varieties show varying degrees of resistance, the Marshall and Marshall-like varieties being highly susceptible.

Possible control measures are suggested and discussed.

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A COMPARATIVE STUDY OF THE GLUTELINS OF THE CEREAL GRAINS[1]

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INTRODUCTION

When in 1908 the American Society of Biological Chemists (*2*)[2] drew up the classification of proteins which has come to be known as the American classification, there was included for the first time a group of proteins designated "glutelin." This group is characterized as consisting of "simple proteins insoluble in all neutral solvents but readily soluble in very dilute acids and alkalies." Recognition of the glutelins was brought about chiefly through the work of Osborne and his collaborators, who found that in the case of many of the cereal grains—wheat, corn, oats, rye, and barley—there remained a fraction of nitrogen after extraction of the grain with water, salt solution, and alcohol. It was assumed that this residual nitrogen, which could be extracted by means of alkaline and acid solutions, represented protein. That this is true was demonstrated in the case of wheat, from which a definite alkali-soluble protein, glutenin, may be readily extracted and prepared in a state of high purity. Chittenden and Osborne (*7, 1891*) and later Osborne and Clapp (*35*) isolated and studied the glutelin of corn. In 1908 Rosenheim and Kajiura (*39*) isolated from rice an alkali-soluble protein which they called "oryzenin."

But despite the fact that this class of proteins was included in the American classification, there has remained considerable doubt whether they are at all widely distributed. As lately as 1924 Osborne (*34*) states that "although it is possible that proteins of the character of glutelins are widely distributed among the different seeds there is no conclusive evidence that this is in fact so." He goes on to say that the only well-defined glutelins are glutenin from wheat, oryzenin from rice, and maize glutelin from maize.

In view of these facts it was deemed desirable to attempt the isolation of proteins of the glutelin type from a number of the cereal grains, and, if success in this were attained, to make a comparative study of the products obtained.

Furthermore, since the glutelins already isolated have been prepared and analyzed by different workers, it seemed highly desirable that as large a number as possible be prepared and analyzed by the same worker, in order that strictly comparable data might be obtained. The work reported in this paper was undertaken with these considerations in mind.

[1] Received for publication Sept. 12, 1927; issued January, 1928. Published with the approval of the director as paper No. 723, Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by R. K. Larmour to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy, Ross Aiken Gortner, major adviser.

[2] Reference is made by number (italic) to "Literature cited," p. 1118.

HISTORICAL

GLUTENIN FROM WHEAT

Glutenin has been known longer and studied more extensively than any other glutelin. The reasons for this are not difficult to find. In the first place, it is probably the most distinctive of the five proteins of wheat flour. It forms, with gliadin, the combination known as gluten, and is generally considered to be the binder which holds the gluten proteins together. Its characteristic property of forming gluten when combined physically with gliadin is the factor responsible for the ability of wheat flour to form a dough from which yeast-leavened bread may be produced.

In the second place, it was long ago suggested that the gliadin-glutenin ratio is largely responsible for the quality of the gluten, and consequently is of the highest importance as a measure of the strength of flour. This naturally led to much investigation of these two proteins, and fairly accurate methods were developed for the quantitative estimation of them. Fleurent (*11*) fixed the optimum at 75 parts gliadin to 25 parts glutenin. Snyder (*42*) found the ideal ratio to be 65: 35, but he also recognized that the quality factor is probably of more importance than the quantity factor. Blish (*4*), however, showed that the ratio is much more nearly constant for different flours than had formerly been supposed. Grewe and Bailey (*18*) examined 17 different flour samples collected from various parts of the United States and Canada. From the data obtained there was no evidence of any substantial variation in the proportion of the gluten proteins present in strong and weak flours. Sharp and Gortner (*41*) and Blish and Sandstedt (*6*) found that only small variations occur in the ratio of glutenin to total protein of the flour.

The early history of the investigations of the proteins of flour has been admirably detailed by Osborne (*33*) and will be passed over lightly. Suffice it to say that the first systematic study of the wheat proteins was made by Ritthausen. The only quantitative chemical method available for comparison of compounds such as proteins was at that time the ultimate analysis. This is of little practical value, and Ritthausen's investigations shed little light upon the subject. Osborne was the next to undertake an extended investigation of the vegetable proteins (*33*). He used the Fischer method of analysis, which enables one to go a considerable distance in making comparisons of various proteins. After the development of the Van Slyke (*43*) method for determining the distribution of nitrogen of the acid hydrolysate into various fractions, it became possible to differentiate still more clearly the two proteins gliadin and glutenin (*4*). Analyses by these methods (*33*, *4*) seem to furnish sufficient evidence to justify the conclusion that glutenin is a chemical entity distinct from gliadin, and not a derivative of the latter through the process of denaturation.

Yet the individuality of glutenin itself has been called into question by Halton (*19*). Through fractional precipitation of glutenin by the addition of acid to its alkaline solution, he prepared at least two proteins. The separation was effected by adding normal hydrochloric acid to the alkaline solution of glutenin until protein separated out in a flocculent condition. This protein was separated. Further addition of acid to the clear filtrate resulted in a second precipitation of protein. The two fractions so obtained were found to be unlike when their racemization curves were studied. Blish (*5*),

in an attempt to repeat this work, met with no success. He found, however, that glutenin, if partially racemized, precipitated at a different hydrogen-ion concentration than did the original glutenin. Since Halton had made no record of the hydrogen-ion concentration at which his precipitation occurred, it was not possible to duplicate his experimental conditions. Blish suggested that a portion of Halton's glutenin may have been racemized, which would account for the two fractions he obtained, and he concludes that glutenin represents only one protein.

The subject has been reopened, however, by Csonka and Jones (*9*), who report the isolation of two glutelin fractions from glutenin. The separation was effected by fractional precipitation from a sodium hydroxide solution by the addition of ammonium sulphate. The differences between the two fractions as shown by analyses were so pronounced that there seems to be little doubt that they represent markedly different substances. The β-glutelin, however, was found to constitute but a relatively small fraction of the total glutenin and, consequently, the value of previous analyses will not be materially lessened for the purpose of comparison unless it be found that the relative amount of the two fractions varies in different flours.

A problem of great importance is that of ascertaining whether or not the glutenin of different sorts of wheat flour varies. This has been attacked from two angles, the chemical and the physical, the latter doubtless being the more precise.

Blish (*4*) prepared samples of glutenin from a high-grade patent flour and from a biscuit flour, and submitted them to the Van Slyke analysis. The results, which are given in Table 13, led him to the conclusion that the two could not be differentiated chemically. Cross and Swain (*8*) carried out a similar investigation, using four widely different types of flour. Their data are also shown in Table 13. An inspection of their results leads to the conclusion that there are marked differences in the chemical constitution of the various preparations. However, they interpreted the data as showing that no distinction can be drawn. It may be stated here that in the opinion of the writer their data are of doubtful value in settling this question.

The evidence thus briefly discussed points strongly to the conclusion that glutenins from different types of flour are identical.

Evidence of a different sort is presented by Sharp and Gortner (*41*) as a result of their investigations of viscosity of flour-in-water suspensions. They found that various flours exhibited different changes in viscosity when treated with acids and alkali. It was shown that these differences must be due to the glutenin, since in leaching the flour with distilled water in order to remove the electrolytes, most of the gliadin was also removed, without, however, causing any substantial modification of the imbibitional capacity of the remaining materials. Without going further into the work of Sharp and Gortner, it may be stated that their conclusion was that "there is an inherent difference in the physicochemical properties of the gluten from strong and weak flours and that these differences are due to the colloidal state of the gluten proteins." They state further that "it has been shown that the differences in colloidal properties apparently reside in the protein, glutenin."

From the papers cited in the foregoing dicussion it seems evident that glutenins from various types of flour are indistinguishable by

present methods of chemical analysis, and by means of the measurements of specific rotation, refractive index, and absorption spectra. It seems to be equally evident that the glutenins differ decidedly in respect to colloidal behavior. These two lines of evidence need not be considered opposing. Even though the chemical composition of the proteins were so similar that differences could not be detected by the finest possible analytical technic, very wide variations in colloidal properties might occur.

GLUTELIN FROM RYE

Einhof (*10*) in 1805 made the first study of the rye proteins and considered that there were two, namely, an albumin which coagulated on boiling, and another, soluble in alcohol, which he called "gluten." Von Bibra (*3*) was the first to recognize an alkali-soluble protein in rye. He called this "casein," but did not analyze it.

Ritthausen (*37*) described three proteins which he found in rye, namely, albumin, soluble in water; an alcohol-soluble protein, which he called "mucedin"; and an alkali-soluble protein, "gluten-casein." The last named was obtained by extracting the rye meal directly with very dilute potash water, precipitating with acetic acid, and washing with water, alcohol, and ether. He made two preparations and gave as an average for the analyses the following figures: Carbon, 52.14 per cent; hydrogen, 6.93 per cent; nitrogen, 16.38 per cent; sulphur, 1.06 per cent; and oxygen, 23.49 per cent.

No further work seems to have been done on the alkali-soluble protein of rye until Obsorne (*30*, *31*, *32*) investigated it. He gave the following distribution of the four proteins in the rye kernel:

	Per cent
Leucosin, soluble in water	0. 43
Gliadin, soluble in alcohol	4. 00
Edestin and proteose, soluble in salt solution	1. 76
Insoluble in salt solution	2. 44
	8. 63

The value for the alkali-soluble protein was obtained by difference. He states that—

> since rye flour yielded no gluten on washing with water, the proteid remaining in the meal after extracting with salt solution and dilute alcohol, could be obtained only by extracting the residual meal directly with dilute potash water. All attempts, however, to thus prepare this substance resulted only in the production of small preparations of very variable composition. The gum present in the seed dissolved freely in the alkaline solution and made it impossible by any means yet discovered to thoroughly purify the preparations.

He concluded that since rye flour yielded no gluten, this substance is "partly or wholly, other than glutenin." Osborne here is probably using "glutenin" in the sense of a protein having physical properties similar to wheat glutenin, and not in a classification sense.

A review of the literature since the time of Obsorne's publication has failed to reveal any further work upon the glutelin of rye.

GLUTELIN FROM OATS

There is probably less definite knowledge regarding the proteins of oats than of any other of the cereal grains which have been investigated in this respect.

Norton (*28*) seems to have been the first to leave any record of work upon the proteins of the oat kernel. He recognized three proteins, viz:

1. Albumin, 0.5 to 2.17 per cent, which was taken up from the epidermis (after starch had been mechanically removed by elutriation with slightly ammoniacal water) by boiling with acetic acid. It was precipitated by neutralizing the solution.
2. Casein or "avenine," as it was named by Johnston, 15.76 to 17.72 per cent, dissolved in slightly ammoniacal water and precipitated by acetic acid.
3. Gluten, 1.33 to 2.46 per cent, which was extracted by alcohol.

Von Bibra (*3*) found that no gluten could be obtained from oat flour by kneading with water. He recognized four proteins or nitrogenous substances. These were albumin, 1.24 to 1.52 per cent, precipitated by boiling the cold-water extract of the ground oats; casein, 0.15 to 0.17 per cent, the material separating from the hot alcohol extract on cooling; plant gelatin, 3 to 3.25 per cent, the substance soluble in both hot and cold alcohol; and a nitrogeneous substance insoluble in water and alcohol, 11.38 to 14.85 per cent. Kreusler (*26*) obtained oat gliadin, soluble in weak alcohol, and oat legumin, soluble in very dilute alkali.

After Kreusler's work there seems to have been no further investigation of the oat proteins until Osborne (*29*) undertook a study of them. Osborne concluded that the protein which is extracted from oats by 0.2 per cent KOH solution is in reality an insoluble modification of the globulin. He makes the following statement:

> The proteid extracted, after complete exhaustion of the oats with alcohol of 0.9 specific gravity, by 10 per cent salt solution . . . and that dissolved out by dilute potash . . . have so nearly the same composition as the globulin extracted by salt solution directly, that they may be regarded as originally identical.

Osborne found further that when ground oats are exposed to the action of water only, a large amount of the proteins become insoluble in dilute potash solution, the amount thus rendered insoluble increasing with the duration of the contact with water. One hour's treatment rendered one-half, and 24 hours rendered two-thirds, insoluble in 0.2 per cent KOH solution. The composition of the part soluble in KOH after action of water and removal of the alcohol-soluble protein, was found to be indistinguishable from the globulin soluble in salt solution.

As a result of this investigation, Osborne stated that the proteins present in the oat kernel are: A proteose and an acid albumin, dissolving out in water; a globulin extracted by cold 10 per cent salt solution; and a globulin extracted by means of 10 per cent NaCl solution at 65° C. which separates out from the saline solution upon cooling.

It must, however, be borne in mind that the only means for comparison of proteins available at this time was the elementary or ultimate analysis. It has since been shown that proteins of the most widely varying properties may give elementary analyses which check very closely.

A search of the literature has revealed no further attempt to elucidate this problem until Lüers and Siegert (*27*) undertook to repeat Osborne's work. They prepared the alcohol-soluble protein, two forms of the globulin, one extracted by cold 10 per cent NaCl solution and one extracted by 10 per cent NaCl solution at 65° C., and the alkali-soluble protein. The last named was prepared by extracting the meal with 0.2 per cent cold KOH solution after it had been previously exhaustively extracted with dilute alcohol. The prepa-

rations were submitted to the Van Slyke nitrogen-distribution analysis. Differences undiscovered by the elementary analyses of Osborne were thus revealed. The only significant differences between the avenalin and the alkali-soluble protein were found in the ammonia and the humin fractions. Curiously enough, the sum of these two fractions was the same for both proteins. Leaving the ammonia and humin fractions out of consideration, there was such close agreement between these two analyses that one is justified in concluding that they represent analyses of the same protein in different degrees of purity. Is it to be decided then, on this evidence, that there is no glutelin in the oat kernel? Osborne was inclined to believe that the avenalin is a changed protein, resulting in some way from the presence of water. It is difficult, however, to believe that such profound differences as were shown by two of the preparations would be produced through this agency.

GLUTELIN FROM CORN

Reference to the work on corn is greatly facilitated by the admirable bibliography compiled by Keith (*24*).

Prior to the investigation by Chittenden and Osborne (*7*, *1891*) practically no work had been done upon the proteins of corn. Ritthausen (*38*) mentions "maize-fibrin," a substance soluble in alcohol. Weyl (*44*) stated that the powdered seeds of corn yielded a globulinlike substance soluble in 10 per cent NaCl solution, which after purification by repeated precipitation in water and re-solution in 10 per cent NaCl, coagulates at 75° C. No mention, however, seems to have been made of the alkali-soluble protein.

Chittenden and Osborne (*7*, *1892*) reported that "corn meal, after thorough extraction with salt solution and warm dilute alcohol, yields little proteid matter to dilute solutions of potassium hydroxide (0.2 per cent). The proteins which they reported at that time were three globulins, one or more albumins, and an alcohol-soluble protein.

Osborne and Clapp (*35*), however, isolated a considerable quantity of the alkali-soluble protein. Regarding it they say:

> Of its individuality as a protein substance we, of course, know nothing, for it has heretofore been impossible to obtain any considerable quantity of it in a state of even approximate purity as regards admixture with nonprotein substances.

Since 1907 considerable work has been done on corn proteins, but no record of the isolation and analysis of the corn glutelin has been found.

GLUTELIN FROM BARLEY

Prior to Osborne's work, very little had been done in regard to the proteins of barley. Von Bibra (*3*) names albumin, plant gelatin, and casein as constituents of barley, but gives no particulars concerning them. Presumably the alkali-soluble protein is indicated by the term "casein."

Kreusler made an investigation of the barley proteins, the results of which are given by Ritthausen (*38*). The "gluten-casein," which evidently corresponds to von Bibra's casein, was prepared by cooling the hot alcoholic extract, whereupon a precipitate settled out. This, when purified by boiling with dilute alcohol and fractionally precipitated from solution in acetic acid, had the following composition: Carbon, 53.84 per cent; hydrogen, 7.16 per cent; and nitrogen, 16. 63 per cent.

Osborne (*30, 31, 32*) found that after extraction of the barley flour with 0.5 per cent salt solution, and then repeatedly with hot 75 per cent alcohol, there still remained 41.7 per cent of original nitrogen in the material. He concluded that since the proteins prepared from barley flour are all so similar to those obtained from wheat flour, this seed also contains a considerable quantity of protein soluble only in dilute alkaline solutions. He stated, however, that—

> all attempts . . . to thus prepare it in quantity sufficient to yield preparations of even approximate purity resulted in complete failure.

He adds that—

> the previous extraction of the flour to remove the proteids already described seemed to render to a great extent the remaining proteid insoluble in potash water and only insignificant precipitates resulted on neutralizing the extracts. The barley flour also contained a large quantity of gum which rendered the filtration of the alkaline extract very difficult, as this gum dissolved freely in potash water.

It looks as though this had been accepted as the final word upon the subject, for since that time there has been discovered no attempt to isolate the glutelin from this cereal.

EXPERIMENTAL

The problem presented was the preparation and analysis of the known glutelins of the cereals wheat, *Triticum vulgare;* rice, *Oryza sativa;* corn, *Zea mays;* and oats, *Avena sativa;* and of the unknown glutelins of the cereals einkorn, *Triticum monococcum;* emmer, *T. dicoccum;* durum wheat, *T. durum;* teosinte, *Euchlaena mexicana;* rye, *Secale cereale;* and barley, *Hordeum vulgare.*

NOMENCLATURE

It may be considered that there is no nomenclature for this class of proteins. "Glutenin" has been established by long usage for the glutelin of wheat. "Oryzenin" is a logical name for the glutelin of rice. There remains, however, the necessity for inventing names for the glutelins of these other grains, unless we are to be content to designate them oat-glutelin, rye-glutelin, etc. In selecting suitable names, it is highly desirable to have some distinctive suffix and one that is related in some manner to the generic name of the grain. Accordingly, it is suggested that the glutelins which have been prepared be named as follows:

Source of glutelin	Name suggested
Triticum vulgare.	Glutenin (name in use).
Triticum spelta.	Spelta-glutenin.
Triticum durum.	Duro-glutenin.
Triticum dicoccum.	Dicocco-glutenin.
Triticum monococcum.	Monococco-glutenin.
Secale cereale.	Secalenin.
Avena sativa.	Avenin.[3]
Hordeum vulgare.	Hordenin.
Zea mays.	Zeanin.
Euchlaena mexicana (*teosinte*).	Teozeanin.
Oryza sativa.	Oryzenin (name in use).

In the succeeding portions of this paper, these names will be used in referring to the various proteins.

[3] Abderhalden and Hämäläinen (*1*) used the term "avenin" to designate the mixture of proteins prepared by extracting oats with NaOH solution without previous removal of albumin, globulin, and prolamine. It should be reserved for the glutelin alone.

PREPARATION AND ANALYSIS OF THE PROTEINS

METHOD

In the isolation of glutelins as described by Osborne, the solutions of the protein in 0.2 per cent alkali were filtered clear. Filtering the alkaline solutions was found impracticable in the present experiments, however, on account of the large volumes of liquid necessary in the extraction of some of the proteins. Consequently the supercentrifuge was called into use. Unfortunately, however, for the uniformity of the procedure, the machine went out of order toward the end of the work; so that three of the proteins, monococcoglutenin, hordenin, and oryzenin, had to be treated in a manner different from the others. A detailed description of the methods of preparation is given, however, for the individual proteins.

All extractions were carried out at room temperature, the materials being well protected with toluene.

All nitrogen determinations were made by the Kjeldahl-Gunning method.

Moisture was determined by heating at 102° C. for two hours in a Freas oven at atmospheric pressure. This method gives reproducible results, and checks fairly closely the vacuum-oven procedure. The samples, after drying, were ashed in platinum at approximately 555° C.

The nitrogen distribution was carried out in general as described by Van Slyke (*43*). In all cases the proteins were hydrolyzed with 60 c. c. of 20 per cent hydrochloric acid for 24 hours, and filtered immediately at the end of this time. The analyses were performed on duplicate solutions, A and B, which are so indicated in the tables.

Modifications introduced were as follows: The acid-insoluble humin was filtered off after hydrolysis and its nitrogen content determined. The acid-soluble humin was determined after the ammonia nitrogen determination. The nitrogen in the precipitate of barium phosphotungstate from the solution of the bases, after very thorough washing, was determined by the Kjeldahl method, and its nitrogen content reported as phosphotungstic acid humin nitrogen. In precipitating the bases, the solutions were heated to near the boiling point before adding the hot 15 per cent phosphotungstic acid solution. No immediate precipitation occurred in this case, but upon slowly cooling to room temperature, a very dense crystalline precipitate settled out. Thereafter the solutions were kept for 48 hours in an ice-cooled refrigerator. Arginine was determined by the use of the apparatus described by Holm (*23*).

In making calculations, no corrections were made for the solubility of the phosphotungstates of the bases.

SOURCE OF PROTEINS

The materials used in the preparation of these proteins were the residues from the alcoholic extraction of the prolamines by Hoffman and Gortner (*21*). These residues had been dried in drawers over steam pipes, and then stored in cans. This material was used rather than fresh samples in order to avoid the rather laborious task of removing the alcohol-soluble proteins prior to extraction of the glutelins. Subsequent experience, however, has led to the conclu-

sion that time might have been saved by beginning with fresh grains, since these residues had become so hard, due to desiccation, that it required, in some cases, weeks in order to get them soaked properly.

GLUTENIN FROM WHEAT

Glutenin was prepared by a modification of Osborne's (*33*) method. There was placed 690 gm. of the crude glutenin (residue from Hoffman's extraction of gliadin) in a 5-gallon stoneware jar with 18 to 20 liters of 0.2 per cent sodium hydroxide solution, and this was stirred frequently for one day. After allowing the mixture to stand undisturbed for 24 hours, 12 liters of the clear solution was removed by siphoning, and this was replaced by 12 liters of fresh 0.2 per cent NaOH solution. This procedure was repeated three times, until all the solid material was dispersed.

The solutions as removed were neutralized with 0.2 per cent HCl until the precipitate settled, leaving an absolutely water-clear supernatant liquid. This was considered to be the point of maximum flocculation of the protein. The precipitates thus obtained were collected and redispersed in 5 to 6 liters of 0.2 per cent NaOH solution. As soon as possible after the addition of the alkali, the solution was put through a Sharples supercentrifuge, 40,000 revolutions per minute, at the medium rate of feed. It was then put through again at the slowest rate of feed, which gives the maximum separation attainable with the machine. These solutions did not, even with this treatment, come out absolutely clear, but always showed a slight opalescence. This, of course, would be expected, since the volume of NaOH solution used in the second dispersion was kept as low as possible in order to save time in centrifuging. The solutions were finally diluted by the addition of an equal volume of distilled water and the protein precipitated by the addition of 0.2 per cent HCl.

After allowing the precipitate to settle, the supernatant liquid was siphoned off and 3 to 4 liters of recovered ethyl alcohol (85 per cent) was added. This was stirred frequently for one day and then decanted off. There followed six successive similar washings with 95 per cent ethyl alcohol. This, it was thought, removed all traces of gliadin. Analysis of the alcohol after the fourth washing showed 0.0017 per cent nitrogen. The protein was washed twice more after this and finally four successive times with 1 to 2 liters of ether. It was then filtered on a heavy cloth, dried for 36 hours at approximately 60° C., and finally ground in a ball mill for 5 hours. The white powder so obtained was subjected to a further drying of 48 hours at 50°–60° in a Freas oven. It was analyzed with the following results:

	Per cent
Moisture	0. 99
Ash	1. 01
Nitrogen	16. 31
Nitrogen corrected for ash and moisture	16. 64

Table 1 shows the nitrogen distribution of the glutenin thus prepared.

TABLE 1.—*The nitrogen distribution of glutenin as determined by the Van Slyke method expressed as percentages of total nitrogen*

Nitrogen	A	B	Mean
Ammonia N	14.63	14.93	14.78
Total humin N	1.85	1.55	1.70
Acid insoluble	.64	.41	.52
Acid soluble	.46	.34	.40
Phosphotungstic	.75	.80	.78
Total basic N	17.96	19.65	18.80
Arginine	10.80	11.00	10.90
Cystine	.44	.37	.40
Lysine	5.34	6.32	5.83
Histidine	1.38	1.96	1.67
Total filtrate N	63.60	63.47	63.54
Amino	58.38	57.92	58.15
Nonamino	5.22	5.55	5.39
Total	98.04	99.60	98.82

By reference to the first two entries in Table 13 it will be noted that these figures in Table 1 agree fairly well with those obtained by Blish, except for the histidine, lysine, and nonamino nitrogen of the filtrate. Either the values for the amino nitrogen determinations are too high in the writer's data or they are too low in those of Blish. In respect to the writer's data, it is considered that A is in error in the basic fraction. The values shown under B are more nearly correct than the mean value. As will be shown subsequently, there is no justification for accepting differences in check determinations as great as those given here. Since all the other fractions check very well, it is assumed that loss of material occurred during the concentration of the solution of the bases.

SPELTA-GLUTENIN FROM SPELT

This protein was prepared by extracting 680 gm. of the spelt residue five successive times with 12-liter portions of 0.2 per cent NaOH solution. The duration of the extraction periods was 1, 5, 5, 6, and 11 days, respectively. The solutions as siphoned off were quite clear and yielded only very small precipitates. The five precipitates thus obtained were collected and redispersed in about 7 liters of 0.2 per cent NaOH solution, and within 30 minutes the liquid was put through the supercentrifuge at the slow rate. The liquid came through quite clear, but was a deep orange color. In the bowl of the centrifuge there was left a considerable quantity of almost white material of a gummy nature. Starch thrown out of suspension at 40,000 revolutions per minute forms a dry hard cake on the walls of the centrifuge bowl, and is nearly all found at the lower end of it, but this material was quite soft, even slimy, and extended most of the length of the bowl. It was removed and triturated with alcohol, washed several times with 95 per cent alcohol, and finally with ether. After drying, it was ground in a mortar. There was thus obtained a white powder, which showed upon analysis a nitrogen content of 0.87 per cent.

The clear solution, upon addition of HCl, yielded a light brown precipitate of the protein. This was treated in the manner described under glutenin. Color was extracted by all treatments with alcohol,

but no color appeared in the ether. After drying at 60° C. and grinding in the ball mill, 16 gm. of fawn-colored powder was obtained, which was analyzed, with the following results:

	Per cent
Moisture	6.55
Ash	1.03
Nitrogen	11.29
Nitrogen corrected for ash and moisture	12.21

Table 2 shows the nitrogen distribution of spelta-glutenin.

TABLE 2.—*The nitrogen distribution of spelta-glutenin as determined by the Van Slyke method, expressed as percentages of total nitrogen*

Nitrogen	A	B	Mean
Ammonia N	7.77	8.34	8.06
Total humin N	5.12	4.93	5.02
Acid insoluble	2.76	2.52	2.64
Acid soluble	1.26	1.19	1.22
Phosphotungstic	1.10	1.22	1.16
Total basic N	24.30	24.57	24.44
Arginine	13.11	13.75	13.43
Cystine	.34	.49	.41
Histidine	2.76	2.83	2.80
Lysine	8.09	7.50	7.80
Total filtrate N	62.45	61.68	62.07
Amino	56.49	56.20	56.35
Nonamino	5.96	5.48	5.72
Total	99.64	99.52	99.59

DURO-GLUTENIN FROM DURUM WHEAT

The protein was prepared by extracting 620 gm. of the crude glutelin with 18 to 20 liters of 0.2 per cent NaOH solution, and afterwards the seven successive 12-liter portions in the manner heretofore described. The duration of the extraction periods was 3, 2, 4, 5, 7, 6, 4, and 6 days, respectively. The first two batches of solution were quite clear and were not centrifuged, but all the later ones were put through the supercentrifuge as they were siphoned from the extraction jar. The first five precipitates obtained were combined and worked up in the manner described under glutenin, and the last three were collected and worked up separately.

As in the case of the spelta-glutenin, there was obtained during the supercentrifuging a white gummy residue which was retained. The residues from all the different centrifugings were combined and extracted for 10 days with 8 liters of 0.2 per cent NaOH solution. This was then decanted off and the residue washed with alcohol and ether and dried. The nitrogen content of the residue was found to be 0.62 per cent.

The two batches of protein varied in nitrogen content, No. 1 having 15.5 per cent and No. 2, 14.2 per cent. The material was almost white, and upon analysis gave the following values:

	Per cent
Moisture	1.78
Ash	.28
Nitrogen	15.15
Nitrogen corrected for ash and mositure	15.46

Table 3 shows the nitrogen distribution of duro-glutenin, preparation 1.

TABLE 3.—*The nitrogen distribution of duro-glutenin as determined by the Van Slyke method, expressed as percentages of total nitrogen*

Nitrogen	A	B	Mean
Ammonia N	13.11	13.40	13.25
Total humin N	2.42	2.56	2.49
Acid insoluble	.87	.81	.84
Acid soluble	.84	.74	.79
Phosphotungstic	.71	1.01	.86
Total basic N	21.13	21.28	21.20
Arginine	10.44	11.56	11.00
Cystine	.95	.81	.88
Histidine	6.33	5.36	5.84
Lysine	3.41	3.55	3.48
Total filtrate N	63.26	63.69	63.48
Amino	54.57	54.37	54.47
Nonamino	8.69	9.32	9.01
Total	99.92	100.93	100.42

DICOCCO-GLUTENIN FROM EMMER

In the extraction of dicocco-glutenin 2,640 gm. of the Hoffman residue was used in two separate batches. The solutions as siphoned off were fairly clear and were not supercentrifuged. The first extraction was of 8 days duration, the second 13 days, and the third 9 days, for each batch. At the end of this time the amount of precipitate obtained was negligibly small and the extractions were discontinued. The crude protein obtained from the first extraction of 8 days was combined and worked up as preparation 1, while the combined precipitates of the following two extractions were worked up later as preparation 2. On redissolving, 5 liters of NaOH solution was used. The solution was immediately put through the supercentrifuge twice. In this case no significant amount of gummy material was obtained. There was a small amount of starch thrown out in the lower part of the bowl. The solution as it finally came from the supercentrifuge was practically water-clear and was considered one of the best examples of separation so far obtained. The two preparations analyzed for nitrogen 14.23 and 13.45 per cent, respectively. There was no difference in the appearance of these two preparations, and so far as it could be controlled, the technic of preparation was the same in each case. Preparation 1 was used in the following analyses:

	Per cent
Moisture	1.52
Ash	.83
Nitrogen	14.23
Nitrogen corrected for ash and moisture	14.58

Table 4 shows the nitrogen distribution of dicocco-glutenin, preparation 1.

TABLE 4.—*The nitrogen distribution of dicocco-glutenin as determined by the Van Slyke method, expressed as percentages of total nitrogen*

Nitrogen	A	B	Mean
Ammonia N	11.04	11.07	11.06
Total humin N	4.41	4.84	4.63
Acid insoluble	1.47	1.45	1.46
Acid soluble	1.87	2.44	2.16
Phosphotungstic	1.07	.95	1.01
Total basic N	24.96	24.24	24.60
Arginine	13.03	13.03	13.03
Cystine	.79	.71	.75
Histidine	5.33	4.26	4.80
Lysine	5.81	6.24	6.02
Total filtrate N	59.07	59.59	59.34
Amino	54.68	54.13	54.41
Nonamino	4.39	5.46	4.93
Total	99.48	99.74	99.63

MONOCOCCO-GLUTENIN FROM EINKORN

Einkorn residue to the amount of 1,830 gm. was used in this preparation in one batch. Periods of extraction were 6, 3, 10, and 10 days, respectively. The precipitates as collected were kept under alcohol of approximately 50 per cent strength, which was changed at intervals of 3 or 4 days. This extracted a considerable amount of reddish coloring material. In no case was the precipitate obtained from the 12 liters of solution very great in quantity. After redissolving the collected precipitates, it was found that the supercentrifuge was out of order, and it was decided to try filtering the solution. Previous experience had shown that filtration through ordinary filter paper removed very little of the suspended material. A heavy duck filter cloth was tried, but it was found that in a very short time the rate of filtration became so slow that this method was impractical. A layer of filter paper pulp, 1 to 1½ inches in thickness on a Büchner funnel was then tried, using a suction pump. This method was satisfactory for a small amount of solution, say the first 2 liters, but the mat rapidly became clogged and even in vacuum of 30 mm. Hg the rate of flow was impossibly slow. To overcome this difficulty, recourse was had to the addition of alcohol to the alkaline solution of the protein. An equal volume of 95 per cent methyl alcohol was added, and this solution filtered with reasonable rapidity. It was filtered twice, and the resultant solution was practically water-clear and of light brown color.

The subsequent treatment of this protein was somewhat different from those heretofore described, because time had become a matter of importance. A full description of it will be given, since two other proteins were prepared in the same manner, namely, hordenin and oryzenin.

Dilute acetic acid was added to the clear filtrate, until it was judged that optimum precipitation had occurred. The precipitate was allowed to settle for approximately an hour, the supernatant liquid decanted, and the remainder of the material poured out upon a heavy duck filter cloth and allowed to drain until most of the liquid

had passed off. The cloth was then gathered up in the hands and as much of the remaining liquid as possible was removed by twisting the cloth. The protein was then removed from the cloth with a steel spatula and triturated in a large porcelain mortar with absolute ethyl alcohol. This was allowed to stand with frequent stirring for 24 hours and the process repeated. After three such treatments ether was substituted for alcohol, and it was found that two treatments of 24 hours' duration with ether were sufficient to desiccate the protein to such an extent that it would dry to a powder in the course of 3 to 6 hours at room temperature. When most of the ether had evaporated, the protein was lightly rubbed in a mortar and placed in a Freas oven at 50–60° C., and allowed to dry for 24 hours.

This method of purifying the proteins seems to involve considerable loss. While there was not a great deal of crude protein originally extracted, the amount finally obtained, namely, 5 gm., was disappointingly small. It is probable that precipitation from the alcoholic NaOH solution is not as complete as from the aqueous NaOH solution. This, however, is conjecture, and is a matter for further investigation.

The product obtained was an exceedingly fine powder of light brown color, which upon analysis gave the following results:

	Per cent
Moisture	1. 43
Ash	1. 07
Nitrogen	13. 23
Nitrogen corrected for ash and moisture	13. 57

Table 5 shows the nitrogen distribution of monococco-glutenin.

TABLE 5.—*The nitrogen distribution of monococco-glutenin as determined by the Van Slyke method, expressed as percentages of total nitrogen*

Nitrogen	A	B	Mean
Ammonia N	10. 80	10. 76	10. 78
Total humin N	6. 03	6. 05	6. 04
Acid insoluble	2. 69	2. 68	2. 69
Acid soluble	2. 30	2. 24	2. 27
Phosphotungstic	1. 04	1. 13	1. 08
Total basic N	20. 09	20. 22	20. 16
Arginine	11. 74	11. 97	11. 86
Cystine	1. 09	1. 00	1. 04
Histidine	2. 47	2. 40	2. 44
Lysine	4. 79	4. 85	4. 82
Total filtrate N	63. 28	63. 25	63. 26
Amino	56. 74	56. 59	56. 66
Nonamino	6. 54	6. 66	6. 60
Total	100. 20	100. 28	100. 24

SECALENIN FROM RYE

In the preparation of this protein a slight departure was made from the ordinary routine. It was thought desirable to ascertain, if possible, whether or not there might be different kinds of protein extracted during the course of the long-continued treatments with alkaline solutions, which seem to be necessary in order to extract the protein material from the residues from the alcoholic extraction.

Accordingly it was decided to collect five separate fractions. The first four fractions were taken off after successive periods of extraction of 24 hours ± 1 hour, while the fifth fraction contained all the protein collected in subsequent extractions. Thus the preparations would result from exposure to the NaOH solution for periods of 1, 2, 3, 4, and 5 to 7 days. In all cases very small quantities of protein were obtained from the solutions as siphoned off from the extraction vessels. The volume of NaOH solution used was so large that no attempt was made to centrifuge at this time. Instead, it was thought advisable to collect the crude precipitates and redissolve them in as small volumes as possible and then centrifuge them very thoroughly. This was accordingly attempted. It was found, however, that in trying to put the collected precipitates back into solution a residue always remained, that is to say, clear solutions such as the original extracts could not be obtained. The mixture was, however, passed through the centrifuge, and it was found that the insoluble portion was more or less completely thrown out of suspension on the walls of the centrifuge bowl. This substance, which was encountered here for the first time in significant quantity, did not look like starch, since it was soft and slimy rather than hard and caked as starch would be after centrifuging. Not much attention was paid to this material at first, but when the same phenomenon was noted with successive fractions, it was thought advisable to keep the material in case it might prove to be protein. A sample of this substance was dried with alcohol and ether and analyzed. It contained 0.21 per cent nitrogen. It was further observed that this material might be dispersed in concentrated alkali solution to form a light brown highly viscous solution or dispersion. This on neutralization gave a flocculent white precipitate which settled very slowly. On the addition of 85 per cent alcohol the material evidently flocculated further, and settled more rapidly. It was washed three successive times with alcohol and finally with ether. After some washings with ether the precipitate had conglomerated and become gellike, so that it could be lifted out by hand. It could be stretched and kneaded like gluten, which, in fact, it resembled considerably. After the ether had evaporated, the exterior of the mass became sticky, and finally dried down to a dark brown horny mass, which ground up to a pure white powder. It was concluded that this material is probably the "gummy substance" referred to by Osborne, and may be responsible for the difficulty hitherto encountered in the preparation of the glutelin of rye.

Unfortunately the significance of the gummy material described above did not become apparent until after most of the fractions of the rye protein had been prepared. Consequently there is no check upon the length of time which elapsed between the redissolving of the crude protein and the centrifuging of it, and it is thought that variations in this period might have been responsible for the varying degrees of purity of the preparations obtained.

The five preparations gave upon analysis a nitrogen content of 12.00, 13.30, 15.15, 13.02, and 11.05 per cent, respectively. In an attempt to redissolve preparation 1 in order to undertake further purification of it, the whole sample was lost. The amounts of protein were small, ranging from 10 to 15 gm. each. The preparations

varied in color from light cream for Nos. 3 and 5, light brown for No. 2, to dark brown for No. 4. Analyses of the four fractions gave the following results:

Preparation	Ash	Moisture	Nitrogen	Nitrogen corrected for ash and moisture
	Per cent	*Per cent*	*Per cent*	*Per cent*
2	0.56	2.19	13.30	13.67
3	.50	2.46	15.15	15.61
4	.38	1.75	13.02	13.30
5	19.16	2.60	11.05	14.12

The preparations 2, 3, 4, and 5 were submitted to the Van Slyke method of analysis, but only the results for 2, 3, and 5 are given in Table 6, since No. 4 was ruined.

TABLE 6.—*The nitrogen distribution of secalenin as determined by the Van Slyke method, expressed as percentages of total nitrogen*

Nitrogen	Preparation 2			Preparation 3			Preparation 5		
	A	B	Mean	A	B	Mean	A	B	Mean
Ammonia N	9.86	9.69	9.78	10.70	10.91	10.80	7.05	7.22	7.14
Total humin N	5.44	5.14	5.29	4.46	4.15	4.30	4.40	4.21	4.30
Acid insoluble	2.18	2.17	2.17	1.53	1.53	1.53	1.44	1.32	1.38
Acid soluble	2.16	1.86	2.01	1.80	1.66	1.73	2.03	2.00	2.01
Phosphotungstic	1.10	1.11	1.11	1.13	.96	1.04	.93	.89	.91
Total basic N	25.54	26.22	25.88	25.40	25.40	25.40	25.79	25.79	25.79
Arginine	13.58	13.73	13.65	13.98	13.74	13.86	13.85	13.75	13.80
Cystine	.60	.33	.47	.70	.65	.68	.63	.56	.60
Histidine	5.19	5.25	5.22	5.11	5.39	5.25	3.67	3.66	3.66
Lysine	6.17	6.91	6.54	5.61	5.62	5.61	7.64	7.82	7.73
Total filtrate N	58.16	58.51	58.34	59.50	59.49	59.49	62.29	62.71	62.50
Amino	51.66	52.21	51.94	52.70	52.78	52.74	57.94	58.35	58.14
Nonamino	6.50	6.30	6.40	6.80	6.71	6.75	4.35	4.36	4.36
Total	99.00	99.56	99.29	100.06	99.95	99.99	99.53	99.93	99.73

AVENIN FROM OATS

Extraction of 8 kgm. of material was carried on in the manner described under glutenin. All the crude precipitates were collected and worked up together. The whole was dispersed in 30 liters of 0.2 per cent NaOH solution and passed through the supercentrifuge twice. Considerable amounts of starch and some gummy material were thus removed. Unfortunately the gummy material was not kept. The resultant solution was fairly clear. It was diluted about 1:1 with distilled water, and the protein precipitated by means of 0.2 per cent HCl solution. The supernatant liquid was decanted off and the residue washed five successive times with 95 per cent alcohol, using about 6 liters per washing. It was finally extracted with 3 liters of ether, drained on a filter cloth, dried in the air for 24 hours, finally over steam pipes for 40 hours, ground in a ball mill, and again dried over the steam pipes for 14 hours. There was

obtained 250 gm. of a light, cream-colored material, which upon analysis gave the following results:

	Per cent
Moisture	2. 04
Ash	1. 41
Nitrogen	15. 23
Nitrogen corrected for ash and moisture	15. 77

Table 7 shows the nitrogen distribution of avenin.

TABLE 7.—*The nitrogen distribution of avenin as determined by the Van Slyke method, expressed as percentages of total nitrogen*

Nitrogen	A	B	Mean
Ammonia N	11. 91	11. 82	11. 87
Total humin N	3. 67	3. 38	3. 53
Acid insoluble	1. 50	1. 19	1. 35
Acid soluble	1. 14	1. 32	1. 23
Phosphotungstic	1. 03	. 87	. 95
Total basic N	24. 71	24. 04	24. 37
Arginine	15. 50	15. 47	15. 48
Cystine	. 93	1. 28	1. 10
Histidine	3. 24	3. 21	3. 23
Lysine	5. 04	4. 08	4. 56
Total filtrate N	58. 89	60. 99	59. 94
Amino	56. 65	58. 79	57. 72
Nonamino	2. 24	2. 20	2. 22
Total	99. 18	100. 23	99. 71

ZEANIN FROM MAIZE

Three kgm. of crude zeanin, residue from the extraction of zein from corn gluten was extracted seven successive times with 0.2 per cent NaOH solution. Collected precipitates were redissolved in 0.2 per cent NaOH, supercentrifuged, and washed with alcohol and ether in the usual manner. An analysis of this product, which was a light cream in color, gave the following results:

	Per cent
Moisture	1. 53
Ash	4. 70
Nitrogen	13. 58
Nitrogen corrected for ash and moisture	14. 49

Table 8 gives the nitrogen distribution of zeanin. These results represent two separate analyses. B and C were run concurrently.

TABLE 8.—*The nitrogen distribution of zeanin as determined by the Van Slyke method, expressed as percentages of total nitrogen*

Nitrogen	A	B	C	Mean
Ammonia N	11. 66	11. 07	11. 24	11. 32
Total humin N	2. 76	2. 45	2. 54	2. 58
Acid insoluble	1. 18	1. 02	1. 06	1. 09
Acid soluble	. 80	. 71	. 87	. 79
Phosphotungstic	. 78	. 72	. 61	. 70
Total basic N	19. 66	18. 31	18. 57	18. 84
Arginine	9. 40	9. 66	9. 29	9. 45
Cystine	. 74	. 99	1. 27	1. 00
Histidine	4. 26	3. 27	4. 18	3. 90
Lysine	5. 26	4. 39	3. 83	4. 49
Total filtrate N	66. 36	67. 54	67. 35	67. 08
Amino	60. 93	61. 67	61. 85	61. 48
Nonamino	5. 43	5. 87	5. 50	5. 60
Total	100. 44	99. 37	99. 70	99. 82

TEOZEANIN FROM TEOSINTE (ANNUAL OR FLORIDA)

Thirty kgm. of teosinte from which the teozein had been removed was extracted in batches of about 2 kgm. each with 0.2 per cent NaOH solution. Four successive extractions of 24 hours duration each were made on each batch. The initial solutions were not centrifuged. The collected precipitates were redissolved in 0.2 per cent NaOH solution and put through the supercentrifuge twice. Considerable amounts of starch were removed, and the resultant solutions were quite clear and very deeply colored. The clear solution was diluted with an equal volume of distilled water and the protein reprecipitated by the addition of 0.2 per cent HCl. It was then dried by treatment with 95 per cent alcohol and ether. Seventy-eight grams of an almost black powder was obtained, which upon analysis gave the following results:

	Per cent
Moisture	1. 60
Ash	2. 39
Nitrogen	10. 54
Nitrogen corrected for ash and moisture	11. 02

Table 9 gives the nitrogen distribution of teozeanin.

TABLE 9.—*The nitrogen distribution of teozeanin as determined by the Van Slyke method, expressed as percentages of total nitrogen*

Nitrogen	A	B	Mean
Ammonia N	10. 32	9. 99	10. 16
Total humin N	8. 31	8. 23	8. 27
Acid insoluble	5. 68	5. 66	5. 67
Acid soluble	1. 61	1. 50	1. 55
Phosphotungstic	1. 02	1. 07	1. 05
Total basic N	26. 63	27. 07	26. 85
Arginine	14. 17	14. 14	14. 15
Cystine	1. 21	1. 16	1. 18
Histidine	7. 89	8. 07	7. 98
Lysine	3. 36	3. 70	3. 53
Total filtrate N	55. 22	54. 73	54. 97
Amino	49. 69	49. 62	49. 65
Nonamino	5. 53	5. 11	5. 32
Total	100. 48	100. 02	100. 25

HORDENIN FROM BARLEY

In the first attempt to extract the glutelin from barley meal, 2 kgm. of the Hoffman residue was treated for 24 hours with 20 liters of 0.2 per cent NaOH solution. After settling, 12 liters of the supernatant liquid was siphoned off and 0.2 per cent HCl solution was added. It was observed that the solution turned milky after the NaOH had been neutralized, but no precipitate settled out, and no amount of adjusting of the acidity of the solution met with any success. It looked as if protein were present, but could not be flocculated, and the attempt at this time was abandoned.

After the other proteins heretofore described had been prepared, another attempt was made to isolate the glutelin from barley. About

2 kgm. of Hoffman's residue was stirred up with 16 liters of 0.2 per cent NaOH solution. Vigorous stirring at frequent intervals was continued for about five hours. After this time, the residue had become extremely viscous. Since the centrifuge was not working, it looked as if it would be impossible to remove the substance which was responsible for the high viscosity of the mixture. In the hope of bringing the liquid into condition to be filtered through paper pulp, a portion was withdrawn and an equal volume of 95 per cent alcohol added to it. A white, fibrous coagulum resulted from the mixing with the alcohol. In the course of an hour this settled, and the clear, slightly colored, supernatant liquid was decanted off and filtered through a heavy layer of filter paper pulp, using very slight suction. A perfectly clear, golden brown solution was obtained. This, upon treatment with dilute acetic acid, yielded a flocculent, light gray precipitate. About 12 liters of the original extract was treated in this manner, the time required being five days. Collected precipitates of the various small batches of filtrate were redissolved, filtered, and reprecipitated. This second filtration gave no clearer solution than the first, and it is doubtful whether any great degree of purification was effected by this operation. The protein was treated in the manner described for monococco-glutenin. Thirteen grams of an almost white powder was obtained. This upon analysis gave the following values:

	Per cent
Moisture	1. 34
Ash	. 69
Nitrogen	15. 30
Nitrogen corrected for ash and moisture	15. 62

The white material which was precipitated by the addition of alcohol to the original extract appeared to be of a gummy nature. It was slightly soluble in cold water, but was readily dispersed in hot water, giving an opalescent suspension which resembled a suspension of soluble starch. This suspension gave a marked starch reaction with iodine, but this was due to traces of starch which must have been very small, since the addition of a very little ptyalin in the form of saliva caused the disappearance of the starch reaction.

Table 10 gives the nitrogen distribution of hordenin.

TABLE 10.—*The nitrogen distribution of hordenin as determined by the Van Slyke method, expressed as percentages of total nitrogen*

Nitrogen	A	B	Mean
Ammonia N	11. 48	11. 28	11. 38
Total humin N	3. 51	3. 02	3. 26
Acid insoluble	1. 42	1. 39	1. 40
Acid soluble	1. 39	1. 05	1. 22
Phosphotungstic	. 70	. 58	. 64
Total basic N	20. 22	20. 28	20. 25
Arginine	10. 88	11. 27	11. 08
Cystine	1. 88	1. 70	1. 79
Histidine	2. 40	1. 70	2. 05
Lysine	5. 06	5. 61	5. 33
Total filtrate N	64. 50	64. 52	64. 51
Amino	57. 69	57. 65	57. 67
Nonamino	6. 81	6. 87	6. 84
Total	99. 71	99. 10	99. 40

ORYZENIN FROM RICE

Two 1,000-gm. batches of polished rice meal which had been extracted with alcohol were treated with 18-liter portions of 0.2 per cent NaOH solution. After 24 hours, 12 liters of the clear supernatant liquid was siphoned off and the protein precipitated by the addition of dilute acetic acid. Three successive extractions of each batch were thus made. The amounts of precipitate obtained were small. The collected precipitates were redissolved and an equal volume of 95 per cent alcohol added. The solution was filtered twice through a mat of filter paper pulp, using slight suction. This gave a perfectly clear, light yellow solution, which upon addition of dilute acetic acid yielded a white flocculent precipitate of the protein. This was dried by means of absolute alcohol and ether. The resultant material required no grinding, but disintegrated to a white fluffy powder. Thirteen grams was obtained. This gave, upon analysis, the following values:

	Per cent
Moisture	1. 97
Ash	. 97
Nitrogen	15. 93
Nitrogen corrected for ash and moisture	16. 41

Table 11 gives the nitrogen distribution of oryzenin.

TABLE 11.—*The nitrogen distribution of oryzenin as determined by the Van Slyke method, expressed as percentages of total nitrogen*

Nitrogen	A	B	Mean
Ammonia N	7. 92	8. 22	8. 07
Total humin N	1. 89	2. 02	1. 95
Acid insoluble	. 53	. 59	. 56
Acid soluble	. 47	. 69	. 58
Phosphotungstic	. 89	. 74	. 81
Total basic N	27. 30	27. 89	27. 59
Arginine	17. 45	18. 46	17. 95
Cystine	. 70	. 53	. 61
Histidine	4. 84	4. 35	4. 60
Lysine	4. 31	4. 55	4. 43
Total filtrate N	63. 00	62. 05	62. 52
Amino	55. 94	55. 99	55. 96
Nonamino	7. 06	6. 06	6. 56
Total	100. 11	100. 18	100. 13

DISCUSSION

Before discussing the results obtained, it might be advantageous to consider the value of the analytical method employed.

The experience gained in the work here recorded has led the writer to believe that the errors most likely to occur in analyses by the Van Slyke method are concerned chiefly with the amino nitrogen determination. Here, very slight errors profoundly affect the values obtained for histidine, lysine, and for the nonamino nitrogen of the filtrate from the bases. The values upon which the highest reliance, in the analytical sense, is placed, are the ammonia nitrogen, humin nitrogen, arginine nitrogen, total basic nitrogen, and total nitrogen

in the filtrate from the bases; in other words, essentially the Hausmann numbers and the arginine value. This does not mean that these are necessarily the true values for the pure protein, but that for the material in hand they can be determined with a high degree of accuracy.

Since the conclusions to be drawn from the analytical data involve certain assumptions regarding the various nitrogen fractions determined, it is necessary here to scrutinize rather carefully the methods used in making these analyses, giving particular attention to what is known regarding the effect of impurities upon the distribution of nitrogen in the various fractions. The humin fraction will be discussed first, because it is the one most profoundly affected by impurities.

Gortner and Holm (*16*), after a long series of experiments (*12–17*) upon humin, concluded that the acid-insoluble humin occurs as a result of interaction of tryptophane and some unknown substance of an aldehydic nature; the acid-soluble humin is due to interaction of tyrosine and the aldehyde, while the phosphotungstic acid humin is probably due to adsorption of monoamino acids on the barium phosphotungstate. Starch and other carbohydrates also affect the humin fraction. This may be accounted for as due partly to the formation of furfural, which acts through its aldehyde group, and partly to the formation of the large amount of "humuslike" material which acts as a nonspecific adsorbent. If this were the complete explanation for the formation of humin neither the ammonia nor the basic nitrogen fractions should be very greatly affected by the presence of impurities, which would increase the humin fraction. The net result of an increase of humin nitrogen would be a corresponding decrease of the amino nitrogen of the filtrate from the bases. But experiment does not bear out these conclusions.

TABLE 12.—*Effect of the presence of carbohydrates on the distribution of nitrogen*

Item	Ammonia N	Humin N	Nitrogen of bases					Nitrogen in filtrate from bases		Total recovery	Observer
			Arginine	Cystine	Histidine	Lysine	Total	Amino	Nonamino		
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	
Lactalbumin	8.57	2.32	7.20	1.30	4.57	12.24	25.31	62.00	2.00	100.20	Osborne, Van Slyke, Leavenworth, and Vinograd (*36*).
7 gm. lactalbumin+7 gm. glucose.	8.37	3.70	8.10	1.05	3.22	12.54	24.91	58.79	3.58	99.35	Do.
3 gm. fibrin	10.15	2.83	10.91	.51	4.36	12.05	27.83	55.43	2.51	98.75	Gortner (*18*).
3 gm. fibrin+9 gm. cellulose.	9.85	7.72	8.56	.71	4.86	11.04	25.17	52.02	3.91	98.67	Do.
Casein	10.27	1.28	7.41	.20	5.76	10.70	24.07	55.81	7.13	98.56	Van Slyke (*43*).
Do	10.37	1.47	7.95	.19	5.95	9.41	23.50	59.32	7.85	102.51	Hart and Sure (*20*)
2.4 gm. casein+12 gm. glucose.	10.09	3.76	7.59	.19	7.31	7.01	22.10	57.85	8.89	102.71	Do.
2.4 gm. casein+12 gm. sucrose.	8.54	9.15	6.77	.19	7.65	6.38	20.99	55.38	9.28	103.38	Do.
2.4 gm. casein+12 gm. starch.	9.20	7.46	6.74	.19	7.30	5.54	19.77	50.89	13.76	101.10	Do.
2.4 gm. casein+6 gm. xylan.	10.40	11.83	5.56	.19	5.52	8.55	19.82	54.98	3.73	100.66	Do.

In Table 12 are given the results of experiments by Osborne et al. (*36*), Gortner (*12*), and Hart and Sure (*20*), showing the effects of added carbohydrates upon the nitrogen distribution of some proteins. An examination of these data shows that in all cases the presence of carbohydrate materially increases the humin nitrogen. The ammonia is significantly affected, however, only when sucrose and starch are added. The presence of the pentosan, xylan, while increasing the humin nitrogen by 700 per cent, has no effect upon the ammonia fraction. This would indicate that the lowering of the ammonia, where it does occur, is not attributable to adsorption on the humin. In order to verify this conclusion, the following experiment was performed:

Two 5-gm. samples of wheat glutenin (the same preparation as analyzed for nitrogen distribution) were weighed out. To one, 2 gm. of cornstarch was added. To the other 1 c. c. of redistilled furfural was added. Each was hydrolyzed for 21 hours with 20 per cent HCl. The acid-insoluble humin was filtered off and washed until free of chlorine ion. The humin precipitates were then treated in the same manner as for the determination of ammonia nitrogen; that is, 150 c. c. water, 100 c. c. 95 per cent alcohol, and an excess of $Ca(OH)_2$ suspension were added to the precipitate in a Claisen flask. This was then distilled at low temperature under suction for 30 minutes. Not a trace of ammonia was obtained by this procedure. The acid-insoluble humin nitrogen increased from 0.52 per cent of the total nitrogen to 1.85 per cent where the starch was added, and to 2.05 per cent where the furfural was added. Evidently, then, the lower ammonia nitrogen observed when carbohydrates are present can not be accounted for by adsorption on the humin. There seems to be only one conclusion possible, namely, that the amide groups are simply not split off. But from a purely chemical viewpoint it seems unreasonable to believe that the presence of substances such as hexoses and pentoses would protect an acid-amide linkage against hydrolysis by 20 per cent HCl. From these facts it might be assumed that the amides are not the only source of ammonia during hydrolysis, but that some amino acids undergo partial deamination. That this actually occurs has been demonstrated by Gortner and Holm (*15*, *16*). Accordingly, it is conceivable that the presence of a large amount of aldehyde, derived from the sugars, might affect the rate of deamination, or the aldehydes, acting as oxidizing agents, might remove one or both hydrogen atoms from these amino groups, with the result that cyclic derivations of the pyrole and pyridine type would result.

Gortner and Holm (*16*) have shown that deamination occurs when pure amino acids are boiled with 20 per cent hydrochloric acid, but that when tryptophane and aldehyde are present there is an increase in the humin nitrogen and a decrease in the ammonia nitrogen. Tryptophane is relatively easily deaminized by boiling hydrochloric acid in the absence of aldehydes, but the humin resulting from interaction of tryptophane and aldehyde is very resistant to acid boiling (*14*). Gortner (*12*) has also shown that when formaldehyde is present in a fibrin hydrolysis "the ammonia nitrogen falls significantly when quantities of formaldehyde are added, but rises very rapidly when larger quantities are present."

Roxas (*40*) suggests the formation of cyclic nitrogen compounds as contributing to the humin nitrogen values. While undoubtedly the true humin nitrogen is largely or wholly derived from tryptophane, such compounds as were postulated by Roxas may be responsible for the formation of a part of the humin formed by hydrolysis in the presence of carbohydrates and may affect the ammonia fraction. However this may be, the liberation of ammonia nitrogen in a protein hydrolysate involves more reactions than simply the hydrolysis of amide linkages, and is one of the many things demanding further investigation. In view of the foregoing considerations, one would not be justified in attaching very great value to the ammonia fraction in comparing proteins which contain any great amount of impurity, unless the nature of the impurities was specifically known.

Turning next to the basic fractions, it is seen that the effect of the presence of carbohydrates is in most instances relatively slight. The results for arginine are somewhat anomalous. Hart and Sure obtained a slight decrease with glucose, while Osborne reports a slight increase. This probably indicates that there is little significant effect. In all of the other cases, however, there is a decrease of arginine nitrogen which can not be ignored. Moreover, the total basic fraction shows a decrease. This can scarcely be explained as due to the decomposition of some of the basic amino acids during hydrolysis, since in that case one would expect an increase of the ammonia fraction. This is not the case. Moreover, Gortner and Holm (*15*) have shown that arginine, histidine, and lysine are very stable to the action of boiling hydrochloric acid and undergo no appreciable deamination even when boiled with hydrochloric acid for six weeks. Neither do the data of Table 12 justify the assumption that the bases are adsorbed on the humin. There is practically equal lowering of total basic nitrogen in the case of starch and of xylan, but the difference in the humin, judged from the values for humin nitrogen, is very great. One other explanation is possible. The phosphotungstates of the bases have a tendency to exist in the colloidal state as a gel. Carbohydrates present might act in the rôle of protectors, preventing the crystallization of the phosphotungstates. If this were the case, it could be predicted that great irregularity would result in the precipitation, under these conditions, of a mixture of amino acid phosphotungstates, since these would all be affected differently by different carbohydrates. An experiment to test this assumption could be carried out readily by treating various aliquots of a known hydrolysate from which humin and ammonia had been removed in the usual manner, with varying quantities of different carbohydrates and then precipitating the bases with phosphotungstic acid. Or it would be better still to start with a known solution of arginine, histidine, and lysine salts.

Effects upon the ammonia, humin, and basic fractions are reflected in the filtrate fraction. Failure of arginine, histidine, and lysine to precipitate will result in an increase of the nonamino nitrogen of the filtrate. This, in general, is what happens, but the increase of the nonamino nitrogen fraction is by far too great to be accounted for in this way. There seems to be no explanation for these data, especially in view of the anomalies presented in the case where xylan is present. There the nonamino filtrate nitrogen drops to less than one-half the normal figure. Does this mean that the prolines were

adsorbed on the humin? How is this to be reconciled with the preceding data? Assuming that these analyses are correct, it appears to be utterly impossible to adduce any explanation which would fit all the facts, but from all the data presented in Table 12, there may be drawn the general conclusion that the ammonia, and the various basic fractions are least affected by the presence of carbohydrate impurities, while the humin and monoamino fractions may be profoundly changed.

The data just discussed, with the exception of the lactalbumin, have all been drawn from mixtures in which the carbohydrate has been very high, two to five times greater than the amount of protein present. It seems not unreasonable to assume that with very much smaller amounts of carbohydrate present, the variations from the true values would be smaller. This would be true especially with regard to the basic fractions.

The foregoing considerations have led to the conclusion that in comparing proteins of doubtful purity, the humin and the filtrate fractions are of least value. It is believed, however, that considerable reliance may be placed on the basic fractions. The ammonia nitrogen, while not without value, should not be relied upon as having great accuracy since it may or may not be affected, according to the sort of impurity present.

Before proceeding further it is desired again to call attention to the analyses of the three preparations from rye. The data shown in Table 6 confirm, to some extent, the foregoing conclusions regarding the effect of impurities.

The three preparations were analyzed to determine whether or not the proteins in each were identical. If this proved to be the case, the analyses would serve as an excellent means of judging the relative effect of the impurities present, since the preparations varied in nitrogen content from 13.67 to 15.61 per cent.

Examining the data, it is seen that there is wide variation in the ammonia nitrogen, even in the case of preparations 3 and 5, in which the total humin values are identical.

The arginine values and the total basic nitrogen values are remarkably constant. The cystine values are of the same order, and the variation is no greater than the experimental error involved in dealing with such minute quantities of material by gravimetric methods. The histidine values for preparations 2 and 3 agree well, but the value for preparation 5 is very much lower. This is considered not significant because of the high probability of error in the amino nitrogen determination. The same thing may be said of the non-amino nitrogen of the filtrate. It should not be inferred that the possibility of absolute accuracy with the Van Slyke apparatus is called into question. It is simply admitted that the technic used is not under as accurate control as would justify the use of these data in finally judging a protein. In the writer's opinion, however, there is presented sufficient evidence here to warrant the conclusion that the proteins of the three preparations are identical. On this assumption the data may now be examined to determine what effect the varying amounts of impurities have had upon the analyses.

Preparations 2 and 3 show rather regular differences. The high humin of 2 is accompanied by a corresponding lowering of the

ammonia fraction. Curiously, the sum of the amide nitrogen and the total humin nitrogen for both 2 and 3 is practically the same, being 15.07 and 15.10 per cent, respectively. Preparation 5, however, is irregular in comparison with the other two. The humin nitrogen is of the same value as that of 3, whereas the amide nitrogen is much lower, being in fact the lowest value for ammonia observed in the whole series of analyses. Since the basic fraction seems to have been, on the whole, relatively unaffected, wide differences necessarily show in the filtrate fraction. These anomalous results may be due to the extraordinarily high ash content, 19.16 per cent, of this preparation. The writer is utterly at a loss to account for this high ash content.

The consideration of these data, together with those of Table 12, seems to justify the conclusion that the arginine and the total basic nitrogen fractions may be used with safety in comparing proteins of doubtful purity. Standards for purity of protein after all are vague and for the most part very empirical.

TABLE 13.—*Nitrogen distribution of some of the cereal glutelins, determined by the Van Slyke method*

Source	Analysis by	Ammonia N	Humin N	Nitrogen of bases					Nitrogen in filtrate from bases		Total recovery
				Arginine	Cystine	Histidine	Lysine	Total	Amino	Nonamino	
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
T. vulgare (patent)	Blish (*4*)	16.50	1.84	9.69	0.18	5.47	2.61	17.95	53.59	9.52	99.40
T. vulgare (biscuit)	do	16.17	1.66	9.27	.18	7.59	1.90	18.94	53.38	9.35	99.50
T. vulgare (Idaho)	Cross and Swain (*8*)	15.62	1.95	10.10	.72	7.06	4.74	22.62	54.02	3.66	97.87
T. vulgare (patent)	do	15.98	1.80	8.17	.71	9.22	4.93	23.03	54.57	3.81	99.19
T. vulgare (club)	do	14.17	2.77	9.23	.65	11.42	4.72	26.02	56.55	2.64	102.15
T. vulgare (forty-fold)	do	13.11	2.60	12.94	.65	6.20	6.42	26.21	55.62	3.98	101.52
T. vulgare (patent)	Hoffman and Gortner (*22*)	13.56	1.44	11.96	.67	4.72	4.52	21.87	53.47	7.50	97.84
Do	Larmour	14.78	1.70	10.90	.40	1.67	5.83	18.80	58.15	5.39	98.82
T. durum	do	13.25	2.49	11.00	.88	5.84	3.48	21.20	54.47	9.01	100.42
T. spelta	do	8.06	5.02	13.43	.41	2.80	7.8.	24.44	56.35	5.72	99.59
T. dicoccum	do	11.06	4.63	13.03	.75	4.80	6.02	24.60	54.41	4.93	99.63
T. monococcum	do	10.78	6.04	11.86	1.04	2.44	4.82	20.16	56.66	6.60	100.24
Avena sativa	do	11.87	3.53	15.48	1.10	3.23	4.56	24.37	57.72	2.22	99.71
Do	Lüers and Siegert (*27*)	12.19	2.89	14.43	1.52	7.24	4.39	27.58	52.35	4.08	99.09
Zea mays	Larmour	11.32	2.58	9.45	1.00	3.90	4.49	18.84	61.48	5.60	99.82
Euchlaena mexicana (teosinte)	do	10.16	8.27	14.15	1.18	7.98	3.53	26.85	49.65	5.32	100.25
Secale cereale (2)	do	9.78	5.29	13.65	.47	5.22	6.54	25.88	51.94	6.40	99.29
Secale cereale (3)	do	10.80	4.30	13.86	.68	5.25	5.61	25.40	52.59	6.90	99.99
Secale cereale (5)	do	7.14	4.30	13.80	.60	3.66	7.73	25.79	58.14	4.36	99.73
Hordeum vulgare	do	11.38	3.26	11.08	1.79	2.05	5.33	20.25	57.67	6.84	99.40
Oryza sativa	Kondo (*25*)	12.36	.66	14.89	.14	4.55	5.94	25.52	* 58.75		97.29
Do	Osborne, Van Slyke, Leavenworth, and Vinograd (*36*)	11.33	1.59	17.17	.46	4.77	4.82	27.22	52.98	6.08	99.20
Do	Larmour	8.07	1.95	17.95	.61	4.60	4.43	27.59	55.96	6.56	100.13

* Total N in filtrate.

To facilitate comparison of the experimental data herein recorded with similar data of other investigators, all available analyses of cereal glutelins are shown in Table 13. No Van Slyke analysis of the glutelin of corn has been discovered. The analyses of glutenin by Cross and Swain have been included despite the fact that they are considered by the writer as of doubtful value.

Attention should be called to the fact that where the various fractions of the humin nitrogen have not been reported, in all probability the total humin nitrogen represents only the sum of the acid-insoluble and acid-soluble humin nitrogen. Therefore the values in total humin nitrogen will always be somewhat lower than those in which is included the phosphotungstic acid humin nitrogen.

A comparison of the writer's results on glutenin with those of Blish, shows great variations in the ammonia nitrogen, the histidine, and the lysine fractions, and in the two filtrate fractions. There is fairly close agreement, however, in the values for arginine, total basic nitrogen, and total nitrogen of the filtrate from the bases. This indicates that there have been differences in the manipulation of the amino nitrogen determination.

Turning next to the analyses of avenin from oats, even greater differences are noticed. The ammonia nitrogen in this case checks very well, as does also the humin nitrogen, if allowance is made for the phosphotungstic acid humin fraction. The arginine nitrogen is higher than that of Lüers and Siegert, while the total basic nitrogen is lower. This, of course, throws the histidine values out of agreement. It is a surprising fact that the lysine values agree. It must be admitted that, on the whole, there is not very good agreement between these two determinations. There might be offered in explanation the fact that the source of the two preparations varied widely, since one was grown in Germany and the other in America. Not much value is attached to this explanation, however.

More satisfactory results were obtained with oryzenin. There is very close agreement with the analysis by Osborne, Van Slyke, Leavenworth, and Vinograd, except for the ammonia nitrogen and the amino nitrogen of the filtrate from the bases. The humin, arginine, cystine, histidine, lysine, and nonamino nitrogen of the filtrate fractions all agree as closely as could be expected for different preparations of the same protein by different workers. The ammonia nitrogen of the writer's analysis is unaccountably low and there is a corresponding increase in the amino nitrogen of the filtrate from the bases. The agreement with Kondo's data is not as good.

These comparisons furnish further evidence for the conclusion previously expressed that the basic fractions should be considered the most reproducible and reliable values in the whole analysis.

In making a comparison of the analyses of all the proteins it would be well to bear in mind the fact that up to the present glutenin, oryzenin, and corn glutelin have been considered the only well-defined representatives of the glutelin class. These then should be useful in adjudging the relationship of the others.

On examining these data one must be led to conclude that there is a very wide range in the degree of purity. The humin fraction varies from 1.70 per cent for glutenin to 8.27 per cent for the glutelin from teosinte. Correspondingly, the nitrogen content of the preparations varies from 16.64 per cent for glutenin to 11.02 per cent for teosinte. The same treatment that was necessary to produce a preparation of wheat glutenin and of oryzenin comparable in purity with those obtained by other workers, failed to give more than a very impure product in the case of most of the other cereal glutelins. This is due doubtless to the presence of gums. Methods more

refined than those in use at present will have to be devised before glutelin preparations of a high degree of purity can be obtained.

The evident impurity of many of the preparations makes it impossible to draw comparisons between the humin values. The same objection applies to the total nitrogen of the filtrate from the bases. The ammonia values may have some significance, but for reasons previously given little reliance can be placed on them. Since the nature of the impurity present in the protein is unknown, one is unable to say what effect it might have had on this fraction. However, the ammonia nitrogen values for all the new proteins fall within the range of values for glutenin and oryzenin as shown by the rearrangement in Table 14.

TABLE 14.—*Values of ammonia nitrogen of the various glutelins, arranged in order of magnitude*

Glutelin	Ammonia N	Glutelin	Ammonia N
	Per cent		*Per cent*
Glutenin	* 16.50 14.78	Dicocco-glutenin	11.06
		Monococco-glutenin	10.78
Duro-glutenin	13.25	Teozeanin	10.16
Avenin	11.87	Secalenin (average)	9.24
Hordenin	11.38	Oryzenin	8.07
Zeanin	11.32	Spelta-glutenin	8.06

* Blish, (4).

TABLE 15.—*Values of arginine nitrogen of the various glutelins arranged in order of magnitude, with corresponding values of total basic nitrogen*

Glutelin	Arginine N	Total basic N	Glutelin	Arginine N	Total basic N
	Per cent	*Per cent*		*Per cent*	*Per cent*
Glutenin (Blish (4))	9.27	18.94	Dicocco-glutenin	13.03	24.60
Zeanin	9.45	18.84	Spelta-glutenin	13.43	24.44
Glutenin (Larmour)	10.90	18.80	Secalenin (average)	13.77	25.69
Duro-glutenin	11.00	21.20	Teozeanin	14.15	26.84
Hordenin	11.08	20.25	Avenin	15.48	24.37
Monococco-glutenin	11.86	20.16	Oryzenin	17.95	27.59

The same thing holds true for the arginine and total basic nitrogen values, although the order is somewhat different from that shown above. Table 15 gives the arginine nitrogen values arranged in order of magnitude and the corresponding values for total basic nitrogen. The latter values, it is seen, fall in the order of the arginine except for monococco-glutenin, the difference between it and hordenin being negligibly small, and avenin, which is three places out.

Summarizing the data, it is seen that glutenin and oryzenin, the best known glutelins, appear to occupy practically the extreme opposite limits of the class in respect to ammonia nitrogen, arginine nitrogen, and total basic nitrogen. Corresponding values for all the other cereal glutelins fall between these limits in all cases. Consequently, if the assumption regarding the reliability of the arginine and total basic values is correct, it must be concluded that all these proteins under discussion possess enough common chemical characteristics to warrant their inclusion in the class with glutenin and

oryzenin. Therefore the writer feels justified in stating that the class of proteins known as glutelins is definite in character, and that proteins of this type exist in all of the cereal grains thus far investigated.

SUMMARY

Alkali-soluble proteins have been prepared from the cereal grains, *Triticum vulgare*, *T. durum*, *T. spelta*, *T. dicoccum*, *T. monococcum*, *Avena sativa*, *Hordeum vulgare*, *Secale cereale*, *Zea mays*, *Euchlaena mexicana* (teosinte), and *Oryza sativa*.

Analyses for nitrogen distribution by the Van Slyke method revealed a well-marked relationship between the various preparations, especially in respect to the basic nitrogen fraction.

Glutenin of wheat, *Triticum vulgare*, and oryzenin of rice, *Oryza sativa*, both well-defined glutelins, occupy positions at the extreme opposite limits of the class in respect to ammonia nitrogen (practically) and total basic nitrogen, and the corresponding values for the other proteins described fall within these limits. This is submitted as evidence that the preparations obtained belong to a definite class of proteins, the glutelins, which is represented in all the cereal grains thus far studied.

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VANILLA ROOT ROT [1]

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THE HOST

The vanilla of commerce (*Vanilla vanilla* (L.) Br., *V. planifolia* Andr.) is cultivated principally in Mexico, Guadeloupe, Venezuela, Tahiti, and Moorea in the Western Hemisphere, and in Madagascar and its dependencies, Ceylon, Mauritius, Reunion, and Seychelles Islands, and Java, in the Eastern Hemisphere. The plant is native to Central America. The cultivation of vanilla on a commercial scale in Porto Rico was begun in 1909 by McClelland (*7*),[2] who demonstrated the possibility of profitable production in that island.

The vanilla climbs by means of "holdfasts," that is, short, slender, rootlike organs arising from the nodes. The holdfasts flatten on the side which comes in contact with a supporting surface and attach themselves to it by means of numerous minute haustoria-like projections. The projections do not behave as haustoria and absorb nutrients from the supporting plant, for they do not penetrate the bark to the cambial tissue. Probably the only function of the holdfasts is that of attachment and support. Plants growing upon dead wood or upon inorganic supports thrive equally with those growing upon living trees. In neither case, however, does the plant grow if its connection with the soil is severed. These conditions are, therefore, seen to be at variance with the statement of Cook and Collins (*4*) that "vanilla is in reality merely an epiphyte or air-plant able to elaborate its food directly from the atmosphere," and also of Newport (*12*) that "the greater part of its nutriment is obtained from the atmosphere by means of its thick, fleshy leaves and tendrils or aerial roots."

The roots arise from the nodes. When a cutting is planted with some of the nodes covered with earth or organic matter, the first roots appear at the covered nodes and the later roots at the exposed nodes. The young roots are very succulent and easily broken. They attach themselves to any available body, elongate rapidly, and exhibit positive geotropism, the tip continuing its downward growth. The holdfasts may be a modified form of root, since they are similar to the roots in habit of attachment and occasionally are transformed into them upon coming in contact with moist, decaying organic matter. A young root which fails to become attached to some surface remains an aerial organ until it reaches the ground and enters the soil. Root hairs, giving the root a white distinctly hairy appearance, grow in abundance where the root is in contact with the soil. The roots

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branch profusely, and the branches are succulent. The roots do not penetrate the soil deeply, and may not enter a heavy clay soil having a moisture-retaining mulch of decaying organic matter. The number of roots produced by a single plant varies greatly with different conditions. One or two roots may arise from a node, and the number of nodes producing roots depends upon the age of the plant and the availability of plant nutrients. Few roots are necessary to supply the needs of a plant grown in a moist, porous soil which is free from disease and well mulched with decaying organic matter. Roots are borne in profusion, however, when the plant is grown under adverse conditions, including lack of moisture, scarcity of organic matter, and a compact, inhospitable soil, or when the established roots have been destroyed.

Like most orchids, vanilla is a shade-tolerant plant and grows in the shade of other plants, which serve the double purpose of screening it from the rays of the sun and supporting the vines. In Porto Rico the dwarf bucare (*Erythrina berteroana*) has been found practicable for carrying vanilla. The blossoms must be hand-pollinated, and the first crop may be obtained in three or four years after planting.

THE DISEASE

HISTORY

A root disease of vanilla was first noticed in Porto Rico in 1918 in the vanillery at the station in Mayaguez. In a short time the planting became so severely infected that it failed to produce a crop, and eventually the plants died. The earliest reference to the disease is that of Thomas (*17*), who isolated a species of Fusarium from diseased roots. What is most likely the same fungus is listed by Stevenson (*16*) as *F. solani* (Mort.) Sacc. McClelland (*8*) in 1922 recorded considerable damage to vanilla by "a root disease, presumably *Fusarium* sp." In the same year Bregger (*3*) noted the occurrence of a root disease which did considerable damage to *V. planifolia*. McClelland (*9*) in 1923 reported that "experimental work under way with vanilla had to be abandoned due to a root disease, presumably *Fusarium* sp." Progress reports on the work herein described were published by the writer (*18*, *19*) in 1924 and 1926. McClelland (*10*) in 1924 stated that growers of vanilla "must consider it a temporary crop because of this root disease."

In the Seychelles Islands, where vanilla was formerly an important crop, the same or a very similar root disease is present, according to a report made in 1921 by Dupont (*5*). In describing the disease he says:

> It seems to me that the roots are first affected. As soon as they reach the ground their tips die off and this die-back eventually kills the whole root system. Aerial roots are next formed in succession to replace the dead roots and it seems on theoretical grounds that the whole energy of the plant is exhausted in producing these organs. As many as three aerial roots have been found to be produced in succession from the same node.

The disease as described above coincides quite closely with that occurring in Porto Rico.

In 1925 Van Hall (*6*) mentioned the occurrence in Java of a root fungus which caused the death of cuttings.

The references cited unquestionably indicate the severity of the disease, and the need for safeguarding the vanilla industry in Porto Rico.

SYMPTOMS

The earliest visible symptom of the disease is the browning and death of the roots. Since many of the roots are in part aerial, their death is rather conspicuous. The underground portion of an infected root dies first. It turns dark brown and decays, and the rot is either soft and watery or somewhat dry, depending upon existing moisture conditions. The progress of the rot is checked at the surface of the soil, and often roots which are apparently healthy will be found to have rotted beneath the surface. The aerial portion of the root slowly shrivels and dies, but it may retain its normal green color for several months after the death of the lower portion. Dying back as the direct result of fungus invasion does not occur, and branches sometimes arise from the aerial portion above an infected region. The branches descend into infected soil and quickly become diseased. Branches springing from infected roots when placed in uninfected soil by the writer made normal growth and were free from disease, indicating that the disease does not always become systemic in the root tissues. Frequently, where soil infection is established, the aerial roots descending from supporting trees are killed before reaching the ground.

With the destruction of the roots the plant ceases growth and devotes its energy to proliferating roots in an attempt to reestablish connections with its food supply. The tender growing tip dies and falls away, the stems and the leaves become a sickly yellow, and the stems shrivel as their reserves of nutrients are removed. The efforts to reestablish roots may continue for a year or longer until the plant exhausts itself and dies. Some diseased plants are enabled to live several years due to their descending roots, which enter the soil and live for a short time before they become infected. Roots 15 feet long have been seen descending the trunks of supporting trees. The older portion of the vine usually dies as it exhausts its capacity for root proliferation. In some instances as much as the lower 10 feet of a vine may rot away, whereas the upper part is still green and continues its precarious existence on the small amount of food obtained through the diseased roots. Recovery is unknown and the length of time from infection to death is dependent upon the size and vigor of the plant and the severity of the infection.

So far as is known, none of the few plantings in Porto Rico is free from the disease. The losses caused by it depend principally upon the age at which the plants become infected. Early infection may cause the death of the plants prior to bearing.

THE CAUSAL ORGANISM

Since beginning the study of the vanilla root disease in 1923 the writer has made several hundred isolations from infected roots. In about 90 per cent of the isolations a Fusarium has been the only organism obtained. The strains taken from different plantings at different seasons of the year were so nearly identical that the same

fungus was regarded as the causal organism. This premise, as will be shown, has been substantiated by inoculations made under controlled conditions.

The Fusarium grows well on a large range of culture media. Steamed rice is the most satisfactory medium for isolating the fungus. Sections of diseased roots, including the margin of the rotted area, were surface-sterilized in a solution made by dissolving 1 gm. of mercury chloride in 500 c. c. of 50 per cent alcohol. They were then washed in sterile water, split open, and placed in tubes of steamed rice. A sterile rod was used to push the sections into the rice. After three or four days a white growth of mycelium was observed arising from the root sections. On rice the initial growth of mycelium is white, and the substratum becomes colored as the age of the culture advances. In 4-weeks-old cultures the color of the substratum varies from white in the most recently invaded sections to light pink and Corinthian pink (Ridgway's Color Standards) (*14*) in the older portions. Microspores are borne very abundantly, but macrospores are less in evidence. At four weeks the macroconidia present were mostly one-septate, and 9.2 to 17.5 microns long, with an average length of 13.5 microns. Two-septate spores were 16.7 to 20 microns long, with an average length of 17.8 microns. Three-septate spores were 26.7 to 35 microns longs, with an average length of 31.6 microns. As the cultures age the rice gradually changes from reddish to deep blue. A strongly aromatic odor is present in all rice cultures.

In order to obtain data on the morphology and physiology of the fungus for comparision with other species for purposes of identification, suggestions of the Wollenweber, Sherbakoff, and their associates (*21*) were followed and, with the exception of stems of Melilotus, Lupinus, and Alnus, which were not available, the media recommended by them were used for the cultivation of the vanilla Fusarium.

On potato tuber cylinders the fungus produced an abundant white, cottony growth in three weeks. Bluish sclerotia were sparingly produced. They were of irregular shape and composed of a compact mass of bluish-tinged hyphae and chlamydospores. Microconidia and chlamydospores were borne abundantly, and only few macroconidia appeared. The chlamydospores were borne in chains, or singly, intercalarily, or terminally on short branches, and varied in diameter from 6.4 to 10.4 microns, with an average diameter of about 8.6 microns. The microspores were cylindrical, guttulate and hyaline, and varied in length from 4.8 to 6.4 microns, and in diameter from 2.4 to 3.2 microns. The mycelium varied in diameter from 1.6 to 6.4 microns, the former young and hyaline, and the latter granular, closely septate, and forming chlamydospores. There was no discoloration of the substratum.

On oatmeal agar, made as recommended by Sherbakoff (*15*), sporodochia were borne in 2-weeks-old cultures. The sporodochia varied in color from white to lilac, those of deeper color being produced near the bottom of the slope. Macroconidia were abundant, nearly always three septate, and varied in length from 28.4 to 42.6 microns. Microconidia also were produced, but chlamydospores were not seen.

On potato-dextrose agar containing 2 per cent of dextrose 2-weeks-old cultures produced numerous white to purple sporodochia. Re-

cently isolated strains caused the agar to redden throughout. This character does not appear in long (2 years) isolated strains. Three-septate macroconidia were abundant. They were densely granular, with a tendency toward constriction at the septae, less distinctly pedicellate and less curved than the normal, and varied in length from 23.4 to 45.9 microns.

On potato-dextrose agar containing 5 per cent of dextrose, numerous deep reddish-purple sporodochia were present. Recently isolated strains cause the medium to turn reddish purple. In long isolated strain cultures the discoloration is confined to the sporodochia-bearing region. Macroconidia were abundant and often many 1 and 2 septate spores were present. The tendency toward constriction of the septae noted in potato-dextrose agar cultures containing 2 per cent of dextrose was intensified, and the normal curvature had disappeared. The macroconidia were blunt and rounded at the ends and certainly abnormal. They varied in length from 15.8 to 36.7 microns.

On *Crotalaria juncea* stems there was abundant white growth in two weeks. No color was observed. Macroconidia were abundant, mostly three septate, and apparently normal in size and shape. They varied in length from 21 to 45.1 microns. Microconidia also were abundant.

Microscopic examination of diseased roots revealed the presence of hyphae in the margins of the diseased and decaying portions, and spore forms in the older portions. The fluctuation in length and septation of macroconidia from inoculated rotting roots from tubes was as follows: 1 septate (very few), 13 to 17 microns; 2 septate (very few), 22 to 32 microns; 3 septate (very numerous), 23 to 55 microns; 4 septate (very few), 32 to 58 microns; and 5 septate (one seen), 60 microns. The macroconidia were hyaline, curved, pedicellate, and somewhat attenuated at the apex, and not constricted at the septae. Dry decaying roots yield chlamydospores occurring singly or in chains of two to four. (Fig. 1.)

Averna-Saccá (*1*), in a morphological description of an unnamed Fusarium which attacks vanilla in Brazil, described the macrospores as—

> fusiformes, com ápices arredondados, rectos ou ligeiramente curvados, hyalinos, (21.6 a 43.2 por 3 a 5.5 microns), primeiro continuos, depois 1–3–4-septados. Em geral, 3 septados.

From the description, the fungus resembles quite closely that in Porto Rico. Averna-Saccá (*1*), however, stated that his Fusarium attacks the leaves and stems, causing small, light, irregular spots, and he did not mention its occurrence on the roots.

Meinecke (*11*) observed an undescribed Fusarium attacking the tender tips and young pods of vanilla vines in Tahiti and Moorea, with the formation of small pustules. He did not find it as a root parasite.

The characters of the macrospores place the vanilla parasite in the Elegans group. In attempting to place the fungus in a previously described species, *Fusarium cubense* EFS. was immediately suggested because of the great preponderance of three septate spores the aromatic odor on steamed rice, and the almost universal infection of

soils in Porto Rico by *F. cubense*. The chlamydospores of the vanilla fungus are somewhat larger than those of *F. cubense* and salmon-colored masses of macroconidia have not been observed in

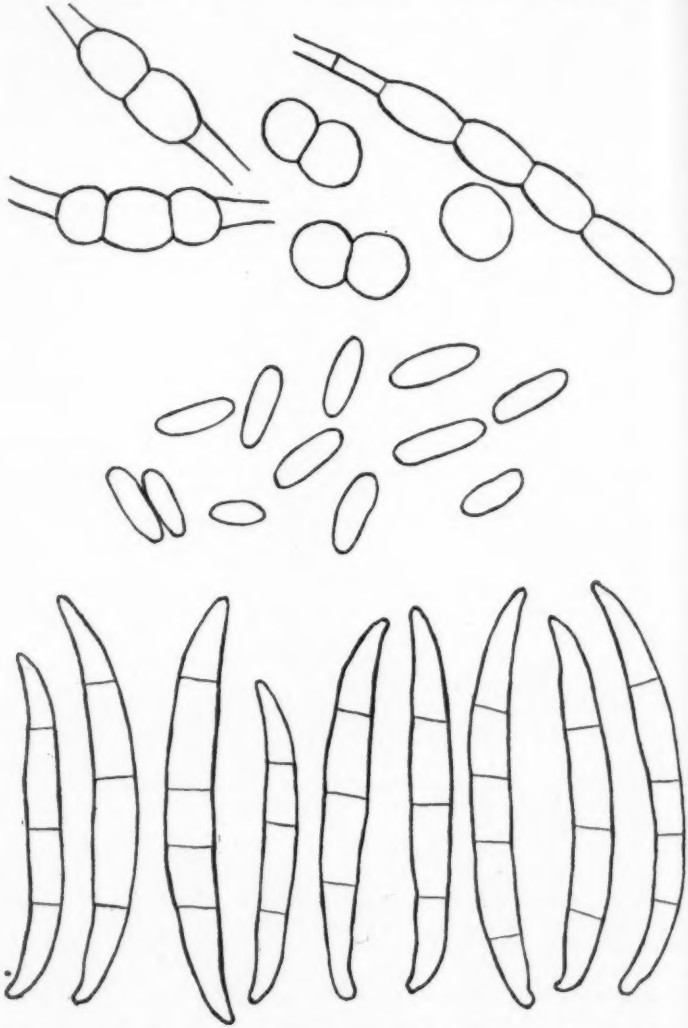

FIG. 1.—*Fusarium batatatis* var. *vanillae* n. var. Macroconidia, microconidia, and chlamydospores in various stages of development. ×1,590

the former, perhaps because of the profuse white mycelial growth commonly obtained. Results of inoculation experiments prove that *F. cubense* is not pathogenic to vanilla.

A culture of the vanilla fungus was sent to C. D. Sherbakoff, who considered it near to Fusarium batatatis Wollenw. (*20*). However, subsequent inoculations of sweet potatoes failed to produce infection, and a culture of *F. batatis* received from L. L. Harter failed to cause infection of vanilla. The Fusaria resemble each other quite closely morphologically, and differ principally in the type of sclerotia produced. Furthermore, the vanilla Fusarium produces sclerotia much less abundantly than does *F. batatis*. The fungi exhibit marked differences in pathogenicity. The vanilla fungus is considered to be *F. batatatis* var. *vanillae* n. var.

Fusarium batatatis var. vanillae, n. var.

Sporodochia borne on decaying vanilla roots. Macroconidia, usually 3 septate, occasionally 1 to 2 septate, rarely 4 to 5 septate. Three-septate spores, 23 to 45 by 2.6 to 4 microns, with an average of 34.2 by 3.6 microns. Macroconidia curved, pedicellate, hyaline, not constricted at septae; apical cell somewhat attenuated. Chlamydospores thick-walled when old, single or in short chains, brown, 6.5 to 10 microns. Microconidia hyaline, oval-elongate, not borne in chains, 4.5 to 7 by 2.2 to 3.6 microns. Blue irregular sclerotia on potato cylinders. In steamed rice cultures the substratum turns vinous red and finally blue. Potato-dextrose agar is reddened, a character which is lost in long-isolated strains. Reddish-purple sporodochia produced on oatmeal and potato-dextrose agar.

Differs from *Fusarium batatatis* Wollenw. in its failure to produce blisterlike sclerotia, which are pushed through the thallus covering the substratum. *F. batatatis* produces sclerotia more abundantly. *F. batatatis* is pathogenic to sweet potatoes and nonpathogenic to vanilla; *F. batatatis* var. *vanillae* is nonpathogenic to sweet potatoes and pathogenic to vanilla.

Parasitic on roots of *Vanilla vanilla* (L.) Br. in Porto Rico.

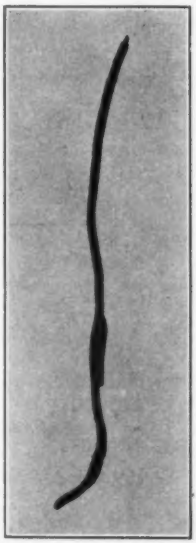

FIG. 2.—Branching of infected aerial root. The branch, if placed in a tube of autoclaved soil, would be likely to develop free from infection

The pathogenicity of the fungus is due to a cytolytic enzyme which it produces, causing the dissolution of the parenchyma tissue of the root. The vascular system is resistant, and cross sections of diseased roots show browning and cytolyzing of the cell walls of the parenchymatous tissues, whereas the cells of the vascular tissues are yet uninvaded. Attempts to isolate the fungus from root sections taken 1 inch above the decaying area have always been unsuccessful. Of course the vascular region is finally invaded and destroyed, but there is never any evidence of the plugging of vessels as in the case of disease caused by *Fusarium vasinfectum* Atk. That there is no general invasion of the root is further indicated by the fact that some diseased roots which are killed back several inches above the soil bear a single branch arising at not more than an inch above the rotted portion. (Fig. 2.) The branch root descends to the soil and quickly becomes infected. In six cases where the writer placed such branches in sterile soil four roots grew normally. In the other two cases, the old root continued to decay and the young root succumbed when the disease reached the point at which the branch arose.

The above facts seem to preclude the possibility that the vanilla Fusarium produces a substance toxic to the plant as does *F. cubense* as shown in the experiments of Brandes (*2*). Brandes observed that cut banana plants wilted when placed in filtrates from cultures in Richards's solution and Uschinsky's solution. His experiments were closely followed by the writer to determine whether the vanilla Fusarium resembled *F. cubense* in this respect. Cultures of *F. cubense* and the vanilla fungus were grown in each solution for two weeks. The fungus was filtered out and cut-bean and cotton seedlings were placed in the filtrates. The results differed somewhat from those obtained by Brandes (*2*), since the wilting occurred in the sterile Richards's solution as well as in the culture filtrates. In Uschinsky's solution Brandes's results were duplicated by the wilting of the seedlings in the filtrates from cultures of *F. cubense*. The filtrate from cultures of the vanilla Fusarium failed to cause wilting, however, even after 18 hours. A study of the symptoms of the disease indicates that absence of a toxic product is to be expected. There is no wilting of the plant beginning with the older leaves as in the case of the banana. The slow dying accompanied by shrinkage of the succulent stems and general yellowing of the entire plant indicates death by starvation.

Proof of Pathogenicity

In order to determine whether the soil was a source of infection, twenty 8-inch pots were filled with soil from a vanillery in which most of the plants had died from root disease. Ten of the pots were autoclaved. One vanilla cutting was planted in each pot, and the pots were placed side by side under partial shade. Six months later the cuttings in the autoclaved pots were making healthy growth, and examination of their roots disclosed no disease. The cuttings in the pots of unsterilized soil made little growth, were yellow, and offered a striking contrast to the plants in the autoclaved pots. All the unsterilized pots contained diseased roots.

In the above-mentioned and in the following experiments, the vanilla cuttings were often taken from diseased vines. No evidence has been found to warrant the view that the disease is transmitted by cuttings except where possibly spores adhere to the surface. To eliminate this factor as a source of infection, all cuttings were dipped for five minutes in a 4:4:50 solution of Bordeaux mixture. This solution kills the tender tips of the plant, but does no serious damage since buds arise readily from the axils of the leaves. Most of the cuttings were 3 to 4 feet long. The supporting stakes were about 5 feet long and, like the cuttings, were dipped in Bordeaux mixture for the further prevention of infection.

In July, 1923, 18 cuttings were planted in autoclaved pots containing coconut fiber. Growth had started on each cutting by September 8, 1923, and the coconut fiber in 12 pots was inoculated with Fusarium grown for one month on moist corn meal. The fungus produced a copious growth and the contents of a liter flask was divided between two pots. The inoculum was mixed with the coconut fiber, care being taken not to injure the vanilla roots. Pots and plants were covered with cheesecloth to prevent the passage of insects acting as carriers.

Monthly observations were made and measurements of the new growth from each cutting were obtained. Table 1 gives the results of the experiment on September 18, 1924.

TABLE 1.—*Results of inoculating vanilla growing in autoclaved coconut fiber with Fusarium batatatis var. vanillae*

Treatment	Total number of plants	Number of diseased plants	Number of healthy plants	Average new growth of diseased plant	Average new growth of healthy plant	Average length of time to appearance of symptoms
				Inches	*Inches*	*Months*
Inoculated	12	7	5	54	109	2½
Control (not treated)	6	3	3	81	94	7½

Table 1 gives little evidence as to the pathogenicity of the organism used for inoculation, since the proportion of diseased plants among the controls is almost as large as among the inoculated. However, the average length of time ensuing between inoculation and the appearance of diseased roots is not without significance, since it varies from two and one-half months in the inoculated plants to seven and one-fifth months in the control plants. The table is of interest chiefly in showing the decreased growth in the diseased plants. The difference is much more marked in the inoculated group on account of the later infections occurring in the control group.

On October 2, 1923, 30 glass tubes, each 26 cm. long and 3½ cm. in diameter, were almost filled with moist coconut fiber, plugged with cotton, and sterilized. Ten tubes were inoculated by mixing corn-meal cultures of the Fusarium with the sterile fiber. In another 10 tubes 12 short sections of diseased vanilla roots were placed on top of the fiber. In a third series, 10 tubes were left to serve as controls. A surface-sterilized vanilla cutting was introduced into each tube so that the basal node would be in contact with the fiber. The tubes were then placed in a frame which held them upright and provided a support for the cuttings. The cuttings rooted quickly from the node in contact with the fiber. They were watered periodically, sterile water being used. The final notes were taken about 13½ months (November 14, 1924) after planting. Unfortunately most of the tops were cut off by a vandal and the data are therefore restricted to root growth and infection.

The accidental infections occurring in the experiment summarized in Table 1 were prevented by growing the cuttings in plugged tubes, but the results obtained in the inoculated tubes were unsatisfactory. Seven of the ten tubes which were inoculated with Fusarium from corn-meal cultures produced diseased roots, but the progress of the disease in the tubes was very different from that under field conditions. In the tubes single root branches became infected and decayed back a short distance, and new branches arose and often attained a length of 6 inches before becoming infected at the tips. In no case was the main root killed. Since only the basal 3 to 4 inches of the cutting were inside the tube, only one or in a few cases two roots entered the fiber from each cutting. The figures on root growth in

Table 2 represent the growth of branches, and the stimulation to branching due to the death of the small roots is shown by the increased root length of the diseased plants. The measurements represent the sums of the lengths of the root branches.

TABLE 2.—*Results of inoculating vanilla in coconut fiber in plugged glass tubes*

Treatment	Total number of plants	Number of diseased plants	Number of healthy plants	Average root growth of healthy plants	Average root growth of diseased plants
				Inches	*Inches*
Inoculated with corn-meal cultures of *F. batatatis* var. *vanillae*	10	7	3	163	191
Inoculated with pieces of diseased roots	10	2	8	125	147
Control (no treatment)	10	0	10	159	

In casting about for an explanation of the unsatisfactory results recorded in Tables 1 and 2, an agar medium was prepared from an infusion of coconut fiber. Inoculations which were made with the vanilla Fusarium on the agar failed to produce growth. Inoculations of the infusion likewise failed to produce growth, although the fungus could be reisolated from the infusion. Coconut fiber is rich in tannin—a chemical inimical to the growth of fungi—and it is probable that the heating of the moist fiber during sterilization liberates sufficient tannin to inhibit the growth of the Fusarium. Since the foregoing inoculations were made in autoclaved coconut fiber, this inhibitory action is believed to account for the uncertainty of infection. Probably the concentration of the tannin was sufficiently high to prevent the growth of the fungus through the substratum and only those roots growing in direct contact with bits of the fungus-bearing corn meal became infected.

Three weeks after tubes of autoclaved moist garden soil were inoculated with the Fusarium the white mycelium of the fungus could be seen throughout. Garden soil was therefore used in all the experiments that followed.

In June, 1924, pots of autoclaved garden soil were planted with vanilla cuttings, one cutting per pot. Inoculations were made November 18, 1924, by stirring into the soil corn-meal cultures of Fusarium which were isolated from vanilla. The pots were partially shaded and were not covered with cheesecloth. Table 3 summarizes the results, which were obtained December 14, 1925.

TABLE 3.—*Results of inoculating vanilla in garden soil*

Treatment	Total number of plants	Number of—							
		Healthy plants after 3 months	Diseased plants after 3 months	Healthy plants after 4 months	Diseased plants after 4 months	Healthy plants after 6 months	Diseased plants after 6 months	Healthy plants after 12 months	Diseased plants after 12 months
Inoculated	50	26	24	24	26	12	38	0	50
Controls (no treatment)	25	21	4	19	6	18	7	13	12

Six months after inoculation the number of aerial roots averaged 3.4 for diseased plants, and 1.8 for healthy plants. This furnishes experimental confirmation of increased root proliferation by diseased plants.

In the experiments, every inoculated plant became diseased, but the high percentage of diseased controls again lays the results open to criticism. Isolations from diseased roots from both inoculated and control plants gave a Fusarium which was apparently identical with that used for inoculation.

A method of inoculation was devised whereby the possibilities of accidental infections were reduced to a minimum. Tubes 26×3½ cm. were two-thirds filled with moist soil, plugged with cotton, autoclaved, and inoculated with Fusarium. Healthy descending aerial roots 6 to 12 inches long were washed with 1:1,000 mercury chloride solution, rinsed with sterile water, and introduced into the inoculated tubes at the side of the cotton plug. The tubes were then tied to the support so as to keep the roots in as nearly their original position as possible. A tarred-paper cover was tied above the cotton plug and between the mouth of the tube and the support to prevent wetting of the plug by rain. (Fig. 3.)

FIG. 3.—Method of inoculating aerial roots of vanilla in situ. The root is washed with mercuric chloride solution and inserted in the tube of autoclaved inoculated soil, which is tied to the support

Inoculations by this method were successful in every instance. Forty-six inoculations resulted in the death of the root, showing symptoms typical of natural infection. The Fusarium was reisolated from each root. Usually, the descending root is invaded and killed as it reaches the soil, and the length of time required for infection varies from 4 to 26 days. With very wet soil the root may come in contact with the fungus on the walls of the tube and die before reaching the soil. (Figs. 4 and 5.) In any case, the roots rot very quickly in infected wet soil. In soil containing only a medium quantity

of water the incubation period is longer. Twenty-five uninoculated control tubes supported normal root growth, the root penetrating to the bottom of the tube and bearing many branches. The control tubes were allowed to remain in place for two months and showed no evidence of infection.

Inoculations of slightly wounded roots always resulted in infection. (Fig. 6.)

A culture of *Sclerotium rolfsii* Sacc., isolated from vanilla roots at the Insular Experiment Station in Rio Piedras by M. T. Cook, was sent to the writer. Ten tubes were inoculated with the fungus and used for inoculating vanilla roots, but no infection was obtained.

To determine the possibility of infecting other portions of the vanilla plant, a water suspension of the Fusarium mycelium and spores was sprayed upon 10 vines. No infection resulted on leaves or stems. Ten stems were inoculated by placing the fungus in a shallow slit in the internodes. Seven days later a brown shrunken area surrounded by a dark green water-soaked margin was observed, and a viscous amber liquid exuded from the wounds. The invaded area enlarged, involving finally the whole internode, but in only two cases did the decay pass a node. The decay continued until

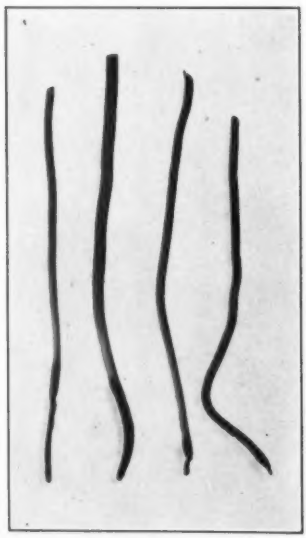

FIG. 4.—Unwounded vanilla roots from tubes of wet soil inoculated with *Fusarium batatatis* var. *vanillae*. Photographed six days after the roots were placed in tubes

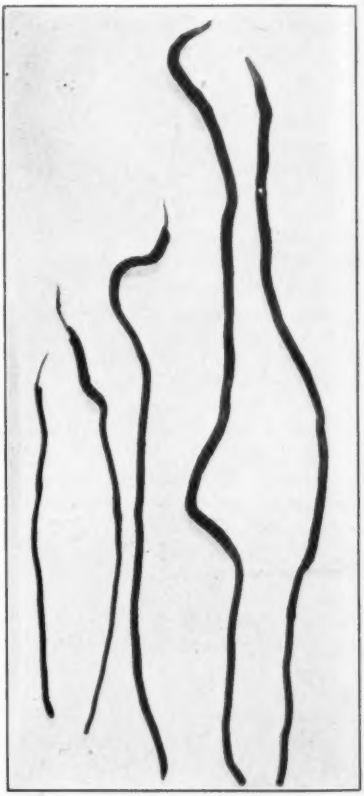

FIG. 5.—Left, two control roots from tubes of autoclaved soil; right, three infected roots from tubes of autoclaved soil inoculated with *Fusarium batatatis* var. *vanillae*. Photographed 12 days after the roots were placed in tubes

it completely destroyed the inoculated internode. (Fig. 7.) The fungus was reisolated from the margins of rotting internodes. A rotting of single internodes is occasionally seen in vanilleries, caused possibly by wound infection. This rot is distinct from the soft rot of the vines as described by Ragunathan (*13*).

Inoculations of wounded stems with *Fusarium cubense* produced no infections, and the wounds healed rapidly and were indistinguishable from those on 10 uninoculated control plants.

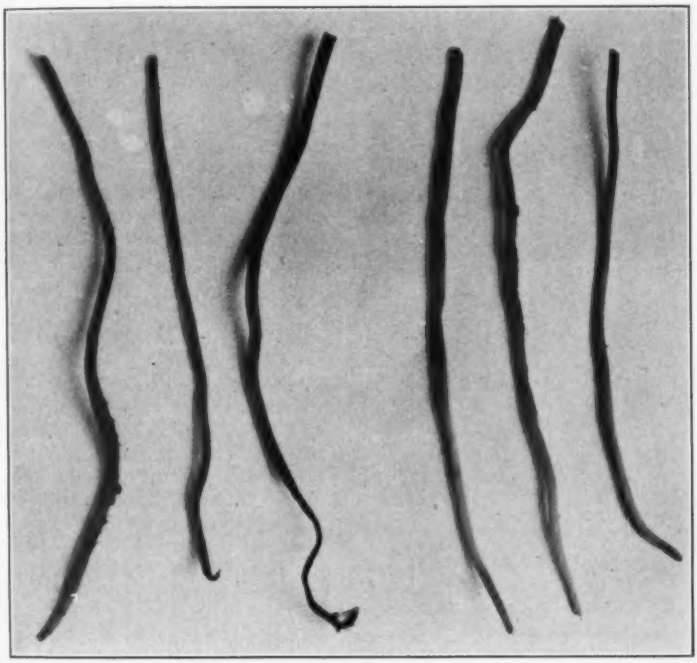

FIG. 6.—Three roots at the left were wound-inoculated with *Fusarium batatatis* var. *vanillae*; the roots at the right were wounded but not inoculated. Photographed five days after inoculation

The Fusarium is able to persist in the soil for at least four years. Cuttings that were planted in a vanillery in places where the plants had been killed by root disease four years previous were attacked and killed in less than a year. U tubes of sterile soil were inoculated in one arm. The average length of time required for the fungus to reach the surface of the soil in the uninoculated arm, a distance of 14 cm., was 10 days. In compact soil the rate of growth would probably be much slower, but the fact that the fungus can thrive in the soil when deprived of a living host indicates long retention of the organism by infected soils.

CONTROL

The problem of controlling vanilla root disease has some features in common with that of controlling banana wilt. Both are caused by a Fusarium which is able to persist in the soil for long periods. Both

hosts are ordinarily propagated by vegetative methods, which offer little opportunity for successful selection for resistance to the disease. Vanilla has the advantage of producing seed from which seedlings may be grown only with difficulty.

In addition to the valuable *Vanilla vanilla* (L.) Br., there are a number of species of Vanilla of low commercial value. One of these, known as the "pom-pom" type, is resistant to root disease. Plants of this vanilla when grown in an infected vanillery where all the *V. vanilla* was killed continued growth for 12 years. The plants are not immune to the disease, since many diseased roots may be found on them, but that they show a marked resistance is apparent from the luxuriant growth and dark green color.

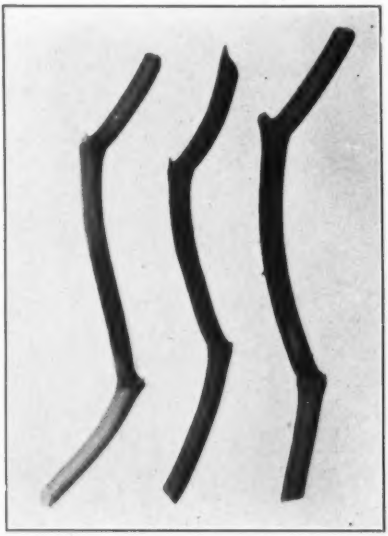

FIG. 7.—Sections of vanilla stems wound-inoculated with *Fusarium batatatis* var. *vanillae*. Photographed three weeks after inoculation.

Crosses between *Vanilla vanilla* and the "pom-pom" vanilla offer a possible means of obtaining resistant strains of high commercial value.

Liming the soil to counteract acidity has been found to be valuable in combating banana wilt. Vanilla thrives best when feeding in a mulch of decaying organic matter. When such a mulch is maintained for several years, however, it is likely to produce a high acid condition, owing to the constant liberation of organic acids on a limited area. No data have been obtained on the effect of liming on vanilla.

McClelland (*10*) has recommended the planting of coffee with vanilla. Coffee thrives in locations that are favorable to vanilla, and the shade trees used as supports for vanilla furnish the shade required for young coffee plants. Coffee is not attacked by the vanilla Fusarium, and vanilla is not susceptible to the coffee root fungi (*Rosellinia bunodes* and an undetermined basidiomycete). Under present conditions the vanilla may be expected to have passed its profitable age after six or eight years, at which time the coffee trees should be producing well.

In the inoculation experiments severe infection by Fusarium was observed to follow a disturbance of the soil or mulch in which the vanilla roots were growing. Vanilla roots are succulent and easily injured, and cultivation under field conditions should be avoided. The shade and mulch should be sufficient to prevent the growth of grass and weeds about the vanilla roots.

SUMMARY

Vanilla vanilla (L.) Br., an orchid producing pods from which the vanilla of commerce is extracted, has been cultivated profitably in Porto Rico.

The roots of the plant are attacked by a root disease, caused by Fusarium sp., which is very severe and widespread. The fungus rots the roots, causing cessation of growth and the gradual death of the plant from exhaustion, due to the proliferation of new roots. These are killed upon reaching infected soil.

The Fusarium is a soil-inhabiting fungus and retains its activity in the soil at least four years and probably much longer.

Sterilized coconut fiber is an unfavorable medium for inoculation, probably because of the presence of tannin.

A method of inoculation of aerial roots in tubes of inoculated soil proved to be very satisfactory for demonstrating the pathogenicity of the fungus, and inoculations invariably resulted in typical infections from which the Fusarium was reisolated.

The vanilla Fusarium is described as a new variety, *Fusarium batatatis* var. *vanillae*. It also resembles *F. cubense* EFS. It is not pathogenic to sweet potatoes, and *F. cubense* and *F. batatatis* Wollenw. are not pathogenic to vanilla.

Crossing *Vanilla vanilla* with a less valuable but highly disease-resistant vanilla is suggested as a possible means of obtaining a disease-resistant strain of high commercial value.

Injury to the roots by cultivation is prejudicial, and mulch and shade should be maintained to prevent the growth of weeds and the necessity for cultivation.

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CORRELATION OF KERNEL TEXTURE, TEST WEIGHT PER BUSHEL, AND PROTEIN CONTENT OF HARD RED SPRING WHEAT [1]

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COMMERCIAL IMPORTANCE OF FACTORS DENOTING QUALITY IN WHEAT

Certain factors in wheat which are now recognized as indicators of milling and baking qualities are becoming increasingly important in determining the market price of wheat. These include grade, kernel texture, test weight per bushel, locality of production, and protein content. Because of the simultaneous occurrence of variations among these factors, it is difficult to compare the effect of each on price. Furthermore, their relative importance changes with the supply of and demand for wheat of the various types and qualities.

Before the development of modern transportation facilities and specialized occupations, when each community produced and prepared for consumption its own food supplies, differences in milling and baking qualities of wheat were of little or no commercial importance. The milling of wheat was then chiefly on a grist-grinding basis. As transportation facilities improved and occupations became more specialized, the grist-grinding trade decreased and the merchandising of wheat and flour increased. This increase in merchandising naturally led to a greater appreciation of the differences in the milling and baking qualities of wheat.

Undoubtedly, flour-yielding capacity was the first factor to be given attention. The plumpness of the wheat kernel was early associated with flour yield, and no doubt the miller was not long in learning that weight per bushel was a good index to plumpness, and, consequently, to flour yield. With the further development of transportation facilities which increased the sources of supplies of raw material and the possibilities for the extension of trade over wider areas, competition between mills increased. Coincident with the increase in competition, baking quality became of greater importance.

At about this time agencies were set up for the inspection or grading of wheat in order to facilitate the trading in that commodity. Inspectors, in accordance with certain rules, determined the grade, which was supposed to be an indication of the condition and milling value of the wheat. This was the beginning of the modern system of grain grading. The rules or specifications for these first grades were indefinite and left much to individual judgment, which varied considerably among the different inspectors. Uniformity in grading was impossible under a system of this kind. To aid in eliminating this condition the trade demanded more definiteness in the grade specifications.

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[2] Valuable advice and assistance on the statistical phase of this work were rendered by H. R. Tolley and Mordecai Ezekiel of this bureau.

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From the first, test weight per bushel was recognized as an important factor in grading. It is probable that gluten quality and quantity also were early recognized as important, but because of the time involved in making a test and the lack of a method whereby uniformity of results among different operators could be assured, these factors were left out of the grade specifications. Kernel texture was used instead. This was accomplished in the hard red spring wheat grades by making a distinction between the "hard" and the "soft" wheat types, and in the hard red winter wheats by the use of the terms "light," "dark," and "medium" in connection with the numerical grade to denote the general color or kernel characteristics of the sample. When the Federal Government established standards for wheat in 1917 this factor was recognized as important in indicating commercial value and was incorporated in the standards.

In harmony with the policy pursued in connection with the other grading factors, definite limitations were placed on the meaning of the terms used to designate the differences in hardness. In the case of the hard red spring and hard red winter wheat classes this was done by establishing subclasses based on the percentage of dark, hard, and vitreous kernels present. This, although accepted by the trade at the time as the best practicable means of designating quality, has never been considered wholly satisfactory, and with the development of improved analytical technic and an increased appreciation of quality, other methods more accurate and definite have been adopted by the trade for determining quality. The protein test is one of these methods, and has become of such commercial importance that a large proportion of the hard red spring and hard red winter wheats are being marketed to-day on that basis in conjunction with grade. Because of this widespread use of protein content in the grading of wheat a study of any relationships existing between it and the other factors used in the grades is of the utmost importance.

The only grading factors associated to any appreciable extent with kernel structure and composition are kernel texture, test weight per bushel, and moisture content. These, therefore, are the only grading factors that have any significant relationship to protein content. It is with these relationships that this paper is directly concerned. Moisture content, however, is taken into account only to the extent of converting all protein data used to the uniform moisture-content basis of 13.5 per cent. For information as to the relationship of the moisture content of wheat to protein content the reader is referred to United States Department of Agriculture Miscellaneous Circular No. 28 (*11*).[3]

CONCLUSIONS OF PREVIOUS INVESTIGATORS

A number of investigators (*2*, *5*, *6*, *9*, *15*)[4] have found that a relationship exists between kernel texture and protein content of wheat. Mangels (*8*), although admitting this relationship, concludes that the percentage of dark kernels can not be considered a reliable index of protein content for the use of the wheat buyer. Shollenberger and Coleman (*12*) found a very definite relationship between kernel

[3] Reference is made by number (italic) to "Literature cited," p. 1151.

[4] Since this article went to press the following paper has been published: COLEMAN, D. A., DIXON, H. B., and FELLOWS, H. C. A COMPARISON OF SOME PHYSICAL AND CHEMICAL TESTS FOR DETERMINING THE QUALITY OF GLUTEN IN WHEAT AND FLOUR. Jour. Agr. Research 34: 241–264. 1927.

texture and protein content when climatic, cultural, and varietal influences were held constant.

As to the relation of test weight per bushel to protein content, Snyder (*15*) found that light weight and shrunken kernels are deficient in starch but comparatively rich in total protein; especially was this evident when the seeds were taken from the same source. Bailey and Hendel (*1*), Davidson and LeClerc (*3*), Mangels and Sanderson (*9*), and Mangels (*8*) found no significant relationship between test weight per bushel and protein content.

METHODS OF STUDY EMPLOYED

So far as known, all previously published results concerning the relationships of kernel texture and test weight per bushel to protein content obtained by correlation studies were derived by gross linear correlation methods; that is, the effect of only one factor at a time was considered, and no attempt was made to determine whether that effect varied in different parts of the range. In the present paper the study of these relationships has been extended, and the results given were derived from the use of net and multiple linear and multiple curvilinear methods. These methods make it possible to consider the effect of several factors working simultaneously and to allow for differences in the effect of each factor in different portions of its range. Moreover, these methods are usually more accurate and complete in their indication of relationships. In cases where the realtionship is not linear or where there is a reversal of direction, curvilinear correlation methods are particularly valuable.

SOURCE AND DESCRIPTION OF DATA

The correlation studies here presented were made from 1,290 samples of hard red spring wheat grown during the crop years 1915 to 1923, inclusive, in 20 Northern and Western States, a few in Canada, and two in Manchuria. Slightly over 60 per cent were produced in the four largest spring wheat-producing States, North Dakota, South Dakota, Minnesota, and Montana. About half of the samples were obtained from commercial lots of grain and the remainder from experiment station plots.[5]

In Table 1 data are given which show by crop year the number of commercial and experimental samples obtained from each of the various geographical sections.

Most of the experimental samples were grown in variety tests by the Office of Cereal Investigations, Bureau of Plant Industry, in cooperation with the various State experiment stations. Twenty-nine varieties are represented in the samples, of which 255 samples were of Marquis and 98 of Kota. In the case of the commercial samples it seems probable, although it has been impossible to ascertain with certainty, that the greater portion of the commercial samples were of Marquis. The commercial wheats were obtained chiefly from Federal grain supervision offices located at the larger grain markets, and are believed to be fairly representative of the hard red spring wheats marketed during each of the respective seasons. Data regarding the varieties of wheat included in the samples are presented in Table 2.

[5] The method employed in securing representative samples is fully described in U. S. Department of Agriculture Bulletin No. 1187 (*15*).

TABLE 1.—*Number and source of samples from hard red spring wheat grown 1915–1923*

Source	1915		1916		1917		1918		1919		1920		1921		1922		1923		Total	
	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples
Arizona																	1		1	0
California					2		2		1		1		2		1		1		10	0
Canada												29				1		6	0	36
Colorado					4				8	1			13		5	5	5	5	35	11
Idaho					2	3	2	2	1		4		1		2		2		14	5
Iowa																10	1	1	1	11
Kansas													3		3				6	0
Manchuria										2									0	2
Michigan							1												0	1
Minnesota					7	5	3	43		9	5	22	9	14	23	41	11	14	63	148
Montana					11		10		9		18	1	27	10	28	2	35	15	138	28
Nebraska					6		4		5	1	3		6	1	4	6	8	10	36	18
North Dakota		1	11		27	53	21	18	13	1	11		25	27	26	3	29	28	163	131
Ohio								33											0	33
Oregon					4	1	2		3	4	7		12	1	6	4	4		38	10
South Dakota					22	20	8		6				9	4	13		11	14	69	38
Utah					1		2		2		1		3			1			9	1
Vermont								2											0	2
Washington					1	13	2		2	3	2		6		5	3	6		24	19
Wisconsin										3		5			2	4			2	12
Wyoming					14		17		5		14	1	15		4		8	1	77	2
Unknown								1		8		7		11		30		39	0	96
Total	0	1	11	0	101	95	73	100	55	32	66	65	131	68	127	110	122	133	686	604

TABLE 2.—*Number of samples of each variety of hard red spring wheat grown 1915–1923*

Variety	1915		1916		1917		1918		1919		1920		1921		1922		1923		Total
	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	Number of experimental samples	Number of commercial samples	
Chul					5														5
Converse													1						1
Early Triumph															1		1		2
Erivan									1		1		1						3
Ghirka					4		3		2		1		1			1			12
Glyndon					10		4		2				1		1				18
Haynes Bluestem		1	1		12	4	9	5	5		5		4		2		5		53
Huston						2					1								3
Iobred																	1		1
Java																	1		2
Kitchener							6		3		1		5		5		4		24
Kota							2		1		5		25		42	4	19		98
Ladoga													1		1		1		3
Larsmie									1		1								2
Marquis			4		18	59	17	36	13		17		30		33		25	3	255
Norka							1				2		5		1		1		10
Pioneer					11		6		6		5		2				1		31
Power					14		11	1	7		6		6		3		7		55
Prelude					11		1		3		5		3						23
Preston			2		14	13	10	1	9		6		7		5		6		73
Progress																	1		1
Red Bobs											4		14		12		13		43
Red Fife						3	1		1		2		2				1		10
Redsask													1		3		6		10
Reliance																	1		1
Ruby							1				4		15		9		9		28
Rysting													1						1
Unknown			4		2		1	13	1	2			6		8		19		56
Probably Marquis						14		44		30		65		68		105		130	452
Total	0	1	11	0	101	95	73	100	55	32	66	65	131	68	127	110	122	133	1,290

Except in test weight per bushel all wheats included in this study met or bettered the requirements of the official standards of the United States for No. 2 grade of hard red spring wheat. The determinations for texture of kernel, test weight per bushel, and other factors used in establishing the grade of these samples were made in accordance with the rules and instructions under which licensed inspectors are required to grade grain.

TABLE 3.—*Correlation between kernel texture and protein content of hard red spring wheat*

Protein content*	Number of samples containing specified percentage of dark, hard, and vitreous kernels																			
	0.1–5 per cent	5.1–10 per cent	10.1–15 per cent	15.1–20 per cent	20.1–25 per cent	25.1–30 per cent	30.1–35 per cent	35.1–40 per cent	40.1–45 per cent	45.1–50 per cent	50.1–55 per cent	55.1–60 per cent	60.1–65 per cent	65.1–70 per cent	70.1–75 per cent	75.1–80 per cent	80.1–85 per cent	85.1–90 per cent	90.1–95 per cent	95.1–100 per cent	Total
Per cent:																					
7	1	2											1								4
8	1	1				2						1									5
9	1	1				2	2	2	2	2	3	2	2	4			2				27
10	2	4	2	2	2	5	5	9	3	12	9	9	5	8	5	8	4	3	3	1	101
11	3		1	1			7	1	7	9	5	11	16	16	16	35	25	23	24	10	210
12		1	1	1		2	4	2	2	4	6	8	3	9	13	29	34	39	29	34	221
13	2	1			1	1		1	1	2	2	3	2	5	7	24	22	32	50	53	209
14		1		1					1	2	1	3	1	2	6	9	11	24	52	79	193
15				1					2		1				3	4	6	7	28	64	116
16		1		1								1			1	1	5	8	16	51	85
17			1											2		2	2	3	16	44	70
18																2	2	1	5	21	31
19																	4	1	5	6	16
20																				2	2
Total	10	12	5	7	5	12	18	15	18	31	27	38	30	46	51	114	117	141	228	365	1,290

$M_K = 80.6 \pm .3925$ $\sigma_K = 20.9 \pm .2775$
$M_P = 13.6 \pm .0432$ $\sigma_P = 2.3 \pm .0305$

* Basis 13.5 per cent moisture.

TABLE 4.—*Correlation between test weight per bushel and protein content of hard red spring wheat*

Protein content*	Number of samples of specified test weight per bushel																				
	43–43.9 pounds	44–44.9 pounds	45–45.9 pounds	46–46.9 pounds	47–47.9 pounds	48–48.9 pounds	49–49.9 pounds	50–50.9 pounds	51–51.9 pounds	52–52.9 pounds	53–53.9 pounds	54–54.9 pounds	55–55.9 pounds	56–56.9 pounds	57–57.9 pounds	58–58.9 pounds	59–59.9 pounds	60–60.9 pounds	61–61.9 pounds	62–62.9 pounds	63–63.9 pounds	64–64.9 pounds	Total
Per cent:																							
7																		2	1	1			4
8																	1	2		2			5
9											1	1		1	2	2	2	3	8	6	1		27
10			1	1				1	1		3		3	5	12	7	11	21	22	11	1	1	101
11					1		1	4	2	3	7	6	7	9	17	21	30	36	33	23	8	2	210
12		1			2	1	1	1	3	3	7	9	9	7	19	33	27	43	31	17	7		221
13			2		1		2	2	3	2	6	6	12	18	19	23	35	22	33	22	1		209
14					2		1	1		3	4	8	6	21	17	27	27	23	27	24	2		193
15					1	1	2	1	1	4	2	3	7	10	12	9	19	18	16	10			116
16								1	2	2	7	3	10	8	8	12	14	9	5	4			85
17								3		3		6	8	3	17	13	4	9	3	1			70
18						1		1		2	4	4	3	3	3	2	6	3	2				31
19										1	1	3	3	3	3	2							16
20												1											2
Total	1	0	3	1	7	3	7	16	12	23	42	50	68	88	128	155	173	190	179	121	20	3	1,290

$M_T = 58.6 \pm .060$ | $\sigma_T = 3.2 \pm .0425$

* Basis 13.5 per cent moisture.

The crude protein (N×5.7) percentages used in connection with these samples are on the basis of a 13.5 per cent moisture content. The determination of this factor for the wheat samples from the crops since 1918 was made by the chemical research laboratory of the Grain Division, Bureau of Agricultural Economics.

Tables 3, 4, and 5, respectively, show the distribution of kernel texture with protein content, the distribution of test weight with protein content, and the distribution of kernel texture with test weight.

TABLE 5.—*Correlation between kernel texture and test weight per bushel in hard red spring wheat*

Test weight per bushel	Number of samples containing specified percentage of dark, hard, and vitreous kernels																			
	0.1–5 per cent	5.1–10 per cent	10.1–15 per cent	15.1–20 per cent	20.1–25 per cent	25.1–30 per cent	30.1–35 per cent	35.1–40 per cent	40.1–45 per cent	45.1–50 per cent	50.1–55 per cent	55.1–60 per cent	60.1–65 per cent	65.1–70 per cent	70.1–75 per cent	75.1–80 per cent	80.1–85 per cent	85.1–90 per cent	90.1–95 per cent	95.1–100 per cent	Total
Pounds:																					
43																				1	1
44																					0
45																			1	2	3
46										1											1
47										1				1		1			1	3	7
48												1							1	1	3
49																	1	2	2	2	7
50										1			1			1	1	1	3	8	16
51															1	3	3	1	3	1	12
52	1									1					2	1	2	3	3	10	23
53	1	1		2										2		6	8	4	3	15	42
54			1									5		1	2	2	6	7	7	19	50
55							2	2	1	2		1	2	1	3	6	6	8	14	20	68
56	2	1	1	2			1	1	1		3	1	1		1	6	9	13	14	31	88
57		1	1			1	1	1	1	2	1	7	2	6	8	16	12	16	17	35	128
58	2	1				1	1	1	4	4	2	2	2	7	6	13	20	14	33	42	155
59	2	3			2	1	2		3	1	4	3	6	9	10	22	11	18	31	45	173
60	2	3	1	1		3	3	6	3	9	6	8	4	7	7	18	19	13	32	45	190
61		2	1		2	5	3	2	2	3	8	7	8	7	6	11	13	19	38	42	179
62				2	1	1	4	2	3	5	2	2	4	3	5	7	6	17	19	38	121
63							1			1	1	1		1		1		3	6	5	20
64														1				2			3
Total	10	12	5	7	5	12	18	15	18	31	27	38	30	46	51	114	117	141	228	365	1,290

CORRELATION OF KERNEL TEXTURE AND TEST WEIGHT WITH PROTEIN CONTENT

The correlation studies of the relationships of the two grading factors kernel texture and test weight to protein content were considered first by gross linear correlation methods,[6] followed by a study employing multiple linear correlation methods (*16*), and a study of these relations by multiple curvilinear methods (*4*).

In Table 6 are given the correlation coefficients and the standard errors of estimate for gross linear correlation, and the correlation coefficients, regression coefficients, and the coefficients of determination (*14*) for net linear correlation found by correlating kernel texture and test weight per bushel with the protein content.

In Table 7 are given the coefficient and index of correlation, standard errors of estimate, and the joint determination of protein content, kernel texture, and test weight as determined by multiple

[6] The coefficients and the standard errors of estimate for gross linear correlation were determined by the use of double frequency tables as described by Richey (*10*)

linear and multiple curvilinear correlation studies. Neither the multiple linear correlation coefficient nor the multiple curvilinear correlation index given in this table can properly be preceded by a plus or minus sign to denote the nature of the correlations because they measure a joint relationship in which the correlation between certain of the factors may be positive and between other factors negative.

TABLE 6.—*Gross and net linear correlation of protein content with kernel texture and test weight*

Item	Gross correlation		Net correlation		
	Coefficient	Standard error of estimate [a]	Coefficient	Regression coefficient	Determination coefficient
Protein content correlated with—					*Per cent*
Kernel texture	+0.5363±0.0134	1.92	+0.5310	+0.0564	27.8
Test weight	−0.2315±0.0178	3.09	−0.2147	−0.1300	4.2
Total					32.0

[a] In percentage points of protein determination.

TABLE 7.—*Multiple linear and multiple curvilinear correlation of protein content with kernel texture and test weight*

Item	Multiple linear correlation			Multiple curvilinear correlation		
	Coefficient	Standard error of estimate [a]	Determination coefficient	Index	Standard error of estimate [a]	Determination coefficient
Protein content correlated with kernel texture and test weight	0.5661	1.87	*Per cent* 32.0	0.6627	1.7	*Per cent* 44.0

[a] In percentage points of protein determination.

The gross correlation coefficient found between kernel texture and protein was +0.5363, indicating a fairly high positive relationship, whereas the gross correlation coefficient between test weight and protein was −0.2315, indicating for these two latter factors a significant negative relationship, although not so close a relationship as that between kernel texture and protein content.

The study of these factors by net linear correlation methods gave +0.531 as the net coefficient of correlation between kernel texture and protein content, and −0.2147 as the net coefficient of correlation between test weight and protein content. These net coefficients indicate practically the same degree of relation as shown by the gross coefficients, and, furthermore, denote that there is no intercorrelation between test weight and kernel texture affecting the individual relations between these two factors and protein. Analysis by means of multiple linear correlation between these two factors and protein gave 0.5661 as the coefficient of multiple correlation, indicating that these two factors combined accounted for 32[7] per cent of the varia-

[7] This figure is derived by squaring the coefficient of multiple correlation (R^2), and represents the proportion of the total variability for which kernel texture and test weight are responsible in this particular combination of factors.

tion in protein content based on the assumption that the relationship is linear. A test for curvilinearity by the approximation method (*4*) revealed, however, that the relationship of both factors to protein was decidedly curvilinear in character. Curves were determined by the curvilinear correlation method to show the net effect of kernel texture upon protein (fig. 1) and the net effect of test weight upon protein (fig. 2). Computing the multiple correlation for these curves

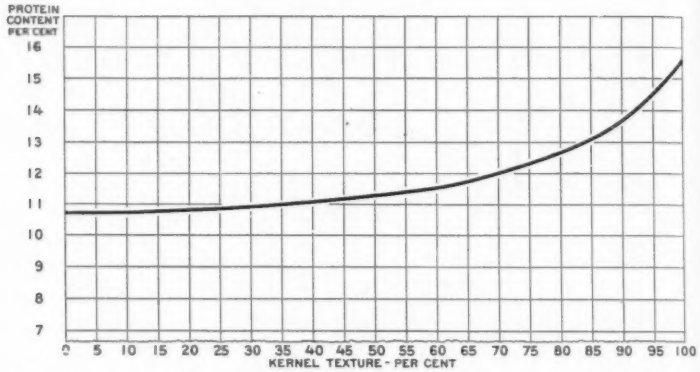

FIG. 1.—Net relation of kernel texture to protein content. This curve shows for hard red spring wheat the differences in protein content due to certain differences in kernel texture. Test weight per bushel was held constant by mathematical methods

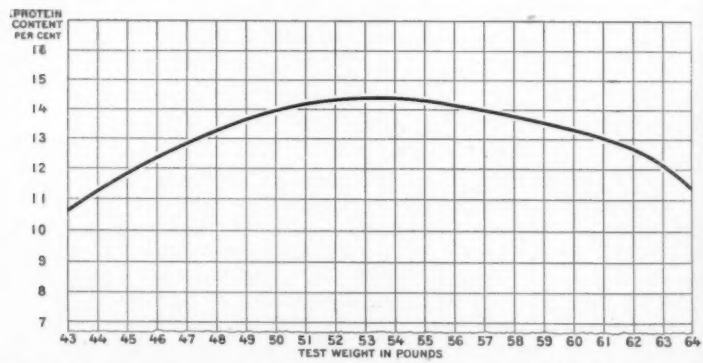

FIG. 2.—Net relation of test weight per bushel to protein content. This curve shows for hard red spring wheat the differences in protein content due to certain diffferences in test weight per bushel. Kernel texture was held constant by mathematical methods

gave a correlation index of 0.6627. This is a considerable increase over the coefficient of multiple linear correlation, 0.5661, showing that there is a marked curvilinearity in the relationships. The joint coefficient of determination for the curvilinear relations is 44, indicating that, when the relations are considered, curvilinear kernel texture and test weight combined account for 44 per cent of the variations in protein content. Since the joint coefficient of determination for the curvilinear relations is 12 points higher than the

joint coefficient for linear correlation, it is evident that the determination of variability of protein has been increased 12 points by curvilinear methods, and that the curves shown in Figures 1 and 2 more accurately describe the relationships of kernel texture and test weight to protein content than would the regression lines of linear correlation.

Although nearly half—44 per cent—of the variability of protein content was due to the influence of kernel texture and test weight, there still remains 56 per cent of the variations which are due to the influence of other conditions or factors. These other factors have not been considered in this article.

RELATION OF KERNEL TEXTURE TO PROTEIN CONTENT

In the correlation studies here reported there was found a fairly strong tendency for protein content to increase as the percentage of dark, hard, and vitreous kernels increased.[8] This tendency, however, was not constant, but was more pronounced among the high percentage of dark, hard, and vitreous kernel groupings than among the intermediate and low percentage groupings of samples. This relationship between kernel texture and protein content is shown in Figure 1. From this graph it may be seen that in the range from 75 to 100 per cent of dark, hard, and vitreous kernels the increase in protein content for each 1 per cent increase in dark, hard, and vitreous kernels is approximately twice as great as that from 0 to 75 per cent. And because 75 per cent marks the dividing line in the Federal wheat standards between the Dark Northern Spring and Northern Spring subclasses of hard red spring wheat, this fact is of particular significance, for it means that the kernel texture limitations of the Dark Northern Spring subclass are about twice as significant as indicators of protein content as those of the Red Spring and Northern Spring subclasses combined.

Below 40 per cent of dark, hard, and vitreous kernels there was for each 1 per cent increase in that factor an average increase of less than 0.01 per cent in the protein content; for each 1 per cent increase between 40 and 75 per cent the average gain in protein content was slightly greater than 0.03 per cent; and for each 1 per cent increase between 75 and 90 per cent an average increase of 0.09 per cent in protein was shown. The greatest rate of increase in protein was found to accompany changes in kernel texture in wheats above 90 per cent of dark, hard, and vitreous kernels. This increase averaged 0.19 per cent gain in protein with each 1 per cent increase in kernel texture.

RELATION OF TEST WEIGHT TO PROTEIN CONTENT

From the correlation studies reported in this article, test weight per bushel was found to have a significant relationship to protein content. The nature of this influence, however, was not the same throughout the entire range in test weight, which extended from 43 to 64 pounds. As the test weight increased in the lower portion of the range, the percentage of protein was also inclined to increase,

[8] As used through this article the terms "increase" and "decrease" or "rate of change" are meant to indicate the differences in the relation of factors from sample to sample. It is obvious that a given sample of wheat can not change its protein content or its proportion of hard kernels. What is meant in the statements in the text is that samples which show the higher precentages of dark, hard, and vitreous kernels also show the higher protein contents.

but as test weight increased in the remainder of the range the percentage of protein was inclined to decrease. As can be seen from Figure 2, wheats up to 54 pounds in test weight per bushel showed about the same relation between differences in protein content and differences in test weight, while above 54 pounds per bushel the protein decreased as test weight increased, this decrease becoming even more marked in wheats weighing above 61.5 pounds.

Wheats with test weight between 43 and 54 pounds increased in protein content on an average 0.34 per cent for each 1 pound increase in test weight. On the other hand, wheats with test weight between 54 and 61.5 pounds decreased on an average 0.20 per cent in protein content with each pound increase in test weight, while wheats with test weight above 61.5 pounds decreased on an average 0.60 per cent in protein content for each pound gain in test weight.

It will be noticed that the increase in protein in wheats weighing between 43 and 54 pounds was nearly twice as great as was the decrease in protein in wheats weighing between 54 and 61.5 pounds. In connection with the decrease in protein content associated with increases in test weight per bushel above 54 pounds, it is interesting to note that up to 61.5 pounds the decrease is only one third as great as that in wheats above 61.5 pounds.

It has been generally believed that there is a negative relation between test weight and protein content; in other words, that the protein content tends to increase as the test weight decreases. Certain previous linear correlation studies have not upheld this belief, but by the application of curvilinear methods it is substantiated within certain limits. (Fig. 2.) These methods show that for wheats weighing more than 54 pounds the relation was negative; for those weighing less than 54 pounds the relation was positive. This reversal of tendency in relationship probably accounts for the lack of relationship reported by previous investigators using linear correlation methods of analysis, because the negative and positive influences operating at opposite ends of the range in test weight tend to cancel each other.

If the reversal in direction of relationship shown by these studies is correct, it should follow that the conditions responsible for lowering the test weights below 54 pounds also tend to lower the protein content, and that the conditions responsible for the test weights above 54 pounds have an opposite effect on the protein content. Because of these opposite effects it is natural to expect that the conditions responsible for them are different. That such is the case is evident from the fact that test weights below 54 pounds seldom occur except in crops injured by rust. Headden (7) reports that rust causes a very marked depression in the protein content of wheat. Therefore, since the extremely low test-weight wheats occur chiefly in the crops affected by rust, and since test weight is, in part at least, a measure of the extent of the rust injury, the correlation between test weight and protein content for wheats thus affected should properly be positive. On the other hand, it has been proved that under normally healthy conditions of growth the proteins are formed or stored in the wheat kernel at an early stage in the kernel's development and the starch is placed there during the later stages. It is the abundance of starch that gives plumpness to the kernel, and plumpness is associated with high test weight; therefore, con-

ditions which arrest the development of the kernel or shorten its period of maturity will produce a kernel of lower starch content and therefore of lower test weight per bushel with a relatively higher proportion of protein content. This, then, accounts for the negative relation between test weights above 54 pounds and protein content, and tends to prove the accuracy of the relationships here shown.

COMBINED RELATION OF KERNEL TEXTURE AND TEST WEIGHT TO PROTEIN CONTENT

As has been shown in previous paragraphs, both kernel texture and test weight were found to have a significant influence upon protein content. Forty-four per cent of the variations in protein were due to the combined influence of these two grading factors.

The results from the linear correlations indicate that kernel texture is much more important than test weight, and the curvilinear correlations, which give a closer approximation to the true relationship, provide evidence that test weight is really considerably more important than was indicated by the linear correlation coefficients. Computation of the coefficients of net curvilinear correlation is a long and tedious operation and was not undertaken.

As previously stated, variations in kernel texture and test weight accounted for 44 per cent of the variations in protein; which means that there still remain 56 per cent of the variations unaccounted for. Undoubtedly the larger portion of these undetermined variations are due to differences in varietal tendencies, soil, cultural methods, quantity of fertilizer, and time of applying it, rainfall, and other growing conditions. These same causes of variation very likely have a direct influence also upon the kernel texture and the test weight. This being the case, these undetermined influences will, through kernel texture and test weight, affect the protein content indirectly as well as directly. What these other influences are and what their importance is, have not been considered in this study.

ESTIMATION OF PROTEIN CONTENT

In the hard red spring and hard red winter wheat-producing sections of this country the price received for wheat by both the country shipper and the terminal-market grain merchant is directly affected by the protein determination made at the terminal market. The farmer, except when he markets his wheat himself or through some cooperative agency, receives no direct benefit from the determination of this factor. There is, however, an indirect way in which the price benefits resulting from protein content may be reflected back to the farmer. Returns from the early shipments for any season may indicate to the country shipper the protein-content tendency in the wheat grown in his locality, and thereby influence the general level of price offered for later deliveries of wheat at that point.

Variations in such factors as soil type and condition, rainfall, elevation, atmospheric temperature, seed variety, seed-bed preparation, and land drainage are responsible for the differences occurring in protein content, and since these factors do not vary as much over small as over large areas, protein returns on a few shipments of wheat are indicative, to some extent at least, of the protein content of other wheat of the same crop produced in the same locality. For a number of years the grain trade has given recognition to this fact by

paying given premiums for wheat that comes from certain shipping points. This is known as "map" buying, and, although it is not always an accurate guide to quality, it serves this purpose in the absence of any better means of judging quality. Under this system the price reflected back to the farmer is based upon the average quality of previous receipts of wheat during that season from his locality. It will be seen that benefits based on this principle may prevent the producer of excellent-quality wheat from obtaining the full market value of his product, and may give to the producer of low-quality wheat more than he is entitled to receive.

Only when the country shipper can know, either through actual test or through some reliable method of estimation, the milling and baking value of the wheat offered to him can the individual producer of high-quality wheat expect to receive the full benefit of the quality of his product. Any means, therefore, by which the shipper can know or more accurately estimate quality will aid in bringing this about. Since protein content has been so generally accepted by the grain trade as an indicator of quality and is being so extensively used for determining the price commensurate with the quality that the wheat is assumed to have, any means whereby the protein content may be estimated by the country shipper would accomplish this purpose.

TABLE 8.—*Average net relation between percentage of dark, hard, and vitreous kernels (kernel texture) and percentage of protein in samples of wheat*

Percentage of dark, hard, and vitreous kernels (kernel texture)	Percentage of protein content	Percentage of dark, hard, and vitreous kernels (kernel texture)	Percentage of protein content	Percentage of dark, hard, and vitreous kernels (kernel texture)	Percentage of protein content
0	10.7	35	11.0	70	12.0
5	10.7	40	11.1	75	12.3
10	10.7	45	11.2	80	12.7
15	10.8	50	11.3	85	13.1
20	10.8	55	11.4	90	13.7
25	10.9	60	11.5	95	14.5
30	10.9	65	11.7	100	15.6

TABLE 9.—*Correction of estimated protein content to take account of differences in test weight*

Test weight per bushel	Correction factor to the protein content given in Table 8	Test weight per bushel	Correction factor to the protein content given in Table 8	Test weight per bushel	Correction factor to the protein content given in Table 8
Pounds	*Per cent*	*Pounds*	*Per cent*	*Pounds*	*Per cent*
43	−2.7	51	+0.9	59	+0.3
44	−2.0	52	+1.0	60	.0
45	−1.5	53	+1.1	61	−.2
46	−.9	54	+1.1	62	−.6
47	−.4	55	+1.0	63	−1.2
48	+.0	56	+.9	64	−1.9
49	+.4	57	+.7		
50	+.7	58	+.5		

From the relationship of kernel texture and test weight per bushel to protein content, which the multiple curvilinear correlation studies reported in this paper have shown, it would appear that a fairly reliable estimation of protein content can be made if kernel texture and test weight per bushel are known. Based on these studies, such a method of estimation is here presented.

In making the estimations of protein content, Tables 8 and 9 are employed. These tables were compiled from the net regression curves shown in Figures 1 and 2.

In Table 8 are presented estimates of the protein value most likely to accompany certain given kernel textures, and in Table 9 are presented correction factors for taking into account the influence of test weight upon protein content. In estimating protein content Tables 8 and 9 are used in the following manner:

Example 1: What percentage of protein in a lot of hard red spring wheat is indicated by a sample of 62 pounds test weight, containing 50 per cent of dark, hard, and vitreous kernels, when the effect of both of these grading factors upon protein content is considered?

Solution: In Table 8, column 1, find 50 per cent kernel texture, read from column 2 the accompanying protein value, which is 11.3 per cent. In Table 2, column 1, find 62 pounds test weight and then, from column 2, read the correction factor, which is -0.6. Make the correction by subtracting 0.6 from 11.3 per cent, which gives 10.7 per cent as the percentage of protein most likely to accompany wheat containing 50 per cent of dark, hard, and vitreous kernels and having a test weight of 62 pounds. In case the correction factor is preceded by a plus sign the estimated protein content is obtained by adding the correction factor to the protein value taken from Table 8.

RELIABILITY OF PROTEIN ESTIMATES

On the basis of the samples used in this study, if one should employ these tables to estimate the protein content of wheat when the kernel texture and test weight are known, the probable error of the estimates would be ± 1.1; that is, one-half the estimates should be within 1.1 per cent lower or higher than the actual protein content. The standard error of estimate would be ± 1.7, and 68 per cent of the estimates should be within 1.7 per cent lower or higher than the actual protein content. That is, in 68 per cent of the samples, if the actual protein percentage was more than the estimate, it did not exceed it by more than 1.7, and if it was less than the estimate, it did not drop more than 1.7. These calculations are based on a uniform moisture content in the wheat.

The kernel textures of the samples used in this study represent the percentage by weight of kernels which were wholly dark, hard, and vitreous in appearance. Kernels of a vitreous character that had starchy spots as well as those that were wholly starchy in appearance were not considered dark, hard, and vitreous. This is in accordance with the interpretation placed on this term as ordinarily used in connection with the inspection and grading of wheat. In this interpretation no account is taken of the relative differences in texture between kernels partly vitreous or wholly vitreous but of a bleached appearance and those wholly starchy in character; and to that extent texture analyses of samples based on this interpretation are not a true indication of kernel texture except in cases where no mottled or bleached kernels are present. Judging from the relationship

found between kernel texture and protein content in connection with this study it is reasonable to believe that if the analysis for kernel texture in these samples had been based on a method more indicative of the true relative hardness of the kernels an even closer relationship would have been apparent, and that the difference existing between the estimated and the actual protein contents would have been materially decreased.

CORRELATION OF KERNEL TEXTURE AND PROTEIN CONTENT WITH TEST WEIGHT

The relationship of kernel texture and protein content to test weight was approached by gross and multiple linear correlation methods. The results of these studies are given in Table 10. Since the correlation of test weight to protein content has already been discussed it need not be considered further.

In correlating kernel texture with test weight a gross coefficient of -0.0953 and a net coefficient of $+0.0351$ were found. These insignificant coefficients show no correlation between these two grading factors.

TABLE 10.—*Gross and net correlation of kernel texture and protein content with test weight*

Item	Coefficient of gross correlation	Coefficient of net correlation	Coefficient of multiple correlation
Test weight correlated with—			
Kernel texture	-0.0953 ± 0.0186	$+0.0351$	0.234
Protein content	-0.2315 ± 0.0178	-0.2147	

SUMMARY

Data from 1,290 representative samples of hard red spring wheat were studied by gross, net, and multiple linear correlation methods and by multiple curvilinear methods to determine the relationship of kernel texture, test weight, and protein content.

A fairly strong tendency was noted for protein content to increase as the percentage of dark, hard, and vitreous kernels increased. This relation was curvilinear in character, and was pronounced in the samples having a high percentage of dark, hard, and vitreous kernels.

A significant correlation was found between test weight and protein content. The tendency was for protein to increase as the test weight increased in wheats weighing less than 54 pounds, but in wheats weighing more than 54 pounds the tendency was for protein to decrease as the test weight increased.

Forty-four per cent of the variation in protein was found to be due to the combined influence of these two grading factors. Kernel texture, however, was considerably more important than was test weight.

A method is presented for estimating the protein content when the kernel texture and test weight are known. By the use of this method the estimated protein content of 68 per cent of the samples would be within 1.7 per cent of the actual protein value.

No correlation was found between test weight per bushel and kernel texture.

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